DETERGENT COMPOSITION

The present invention relates to a detergent composition comprising an endo-glucanase that provides improved detergency performance. The invention also relates to a detergent composition comprising a combination of an endo-glucanase and other enzymes. One aspect of the invention relates to a process of washing a fabric or hard surface with the endo-glucanase-containing detergent.

BACKGROUND OF THE INVENTION

An important objective of a washing process is to transfer, as completely as possible, the soil from the object being washed into the washing solution, such that the soil can then be discarded with the wash solution. A wash process which merely redistributes the soil on the object being washed is obviously not satisfactory. Thus evaluations of detergency performance, i.e. the performance of washing processes, must include consideration both of the removal of soil from the object being washed and of the redeposition of this soil onto the object being washed or onto the equipment being used for the wash process.

With some soils and some surfaces it is very difficult to achieve a satisfactory detergency performance. Problem soils include particulate soils such as clays and carbon. Visible soils are often difficult to wash away completely because they are bound to, or attracted to, a surface by traces of non-visible, sticky substances. Problem surfaces include the surfaces of cotton fibres.

To help overcome such problem soils, detergent formulations may include an anti-redeposition agent. The anti-redeposition agent is added in order to ensure that the above mentioned problem soils, once detached from the fabrics, can be kept dissolved or suspended in the wash liquor in such a way that they are not re-deposited on the cleaned fabric.

To obtain both an anti-redeposition effect and a cleaning effect it has been suggested to add a mixture of cellulases to the detergent, one cellulase having anti-redeposition effect and one having a cellulose-degrading effect. EP 822 973 relates to a detergent composition comprising a mixture of cellulases and optionally also containing additional enzymes. However, there is a need for improved combinations of enzymes having anti-redeposition effect and other enzymes, such as proteases, amylases, hemi-cellulases, lipases and pectinases.

Enzymes are commonly used to remove the sticky substances that increase soil binding. There is a need for improved enzyme performance in terms of soil removal and/or prevention of soil redeposition.

SUMMARY OF THE INVENTION

The invention relates to a detergent composition comprising an anti-redeposition endo-glucanase, i.e. an endo-glucanase having anti-redeposition effect. The inventors have found that such endo-glucanase containing detergents give advantages when used for washing fabrics (especially laundry washing) or hard surfaces (especially automatic dish washing).

In the context of the present invention, an endo-glucanase having anti-redeposition effect is characterised by its ability to prevent redeposition in a wash test.

The inventors also have found that detergent compositions comprising specific combinations of certain endo-glucanase(s) having anti-redeposition effect and certain cellulase(s) having increased stability towards anionic tensides such as linear (straight-chain) alkyl benzene sulfonates (often referred to as "LAS"), have advantages compared to the prior art detergent compositions described in EP 822 973. For instance, a decreased amount of enzyme protein is necessary to obtain the desired cleaning and anti-redeposition effect. This results in improved product economy.

The inventors also have found that it is surprisingly advantageous to include an endo-glucanase having anti-redeposition effect in detergents that also contain other enzymes. These other enzymes include, for example, enzymes that are classified as protease, amylase, beta-glucanase, lipase, hemi-cellulase, cutinase, pectinase and pectate lyase.

Thus in a first aspect the invention relates to a detergent composition comprising an endo-glucanase, wherein the endo-glucanase is selected from one of:

(i) the endo-glucanase having the amino acid sequence of position 1 to position 773 of SEQ ID NO: 2;

(ii) an endo-glucanase having a sequence of at least 90% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2; or a fragment thereof that has endo-glucanase activity;

(iii) an endo-glucanase characterized by the wash test method disclosed herein.

In a second aspect the invention relates to a detergent composition comprising anionic tensides and a combination of an endo-glucanase as defined above and a fungal cellulase, wherein both enzymes are stable in the presence of anionic tensides.

In a third aspect the invention relates to a detergent composition containing both an endo-glucanase as defined above and one or more other enzyme from classes such as, but not limited to protease, amylase, beta-glucanase, lipase, hemi-cellulase, cutinase, pectinase and pectate lyase.

In an aspect the invention relates to a process of using a detergent composition of the invention for washing or cleaning of fabric, hard surfaces or other objects in need of cleaning.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to a detergent composition comprising an endo-glucanase having anti-redeposition effect.

The endo-glucanase with anti-redeposition effect

The endo-glucanase having anti-redeposition effect is according to the invention selected from one of:

(i) the endo-glucanase having the amino acid sequence of position 1 to position 773 of SEQ ID NO: 2;
(ii) an endo-glucanase having a sequence of at least 90% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2; or a fragment thereof that has endo-glucanase activity;
(iii) any endo-glucanase characterized by the wash test method disclosed below.

In a preferred embodiment the endo-glucanase is derived from *Bacillus* sp. AA349, DSM 12648 and also shown in SEQ ID NO: 2 herein or a sequence being 90% identical thereto.

The strain *Bacillus* sp. AA349, which has been isolated from a soil sample originating in Greece, was deposited by the inventors according to the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany, on 25 January 1999 under the deposition number DSM 12648.

The deposit was made by Novo Nordisk A/S and was later assigned to Novozymes A/S.

Detergent compositions comprising anionic tensides

A preferred embodiment the invention relates to a detergent composition comprising anionic tensides, and to a combination of an endo-glucanase as defined above and a fungal cellulase, wherein both enzymes are stable in the presence of anionic tensides, such as LAS.

For the purpose of the present invention, enzymes that are stable in LAS are defined by a LAS stability test. Enzymes that give a LAS stability result of at least 50% are regarded as being LAS stable.

Detergents commonly contain more than one type of surfactant, for example combinations of both anionic and nonionic surfactants. In a preferred embodiment the invention relates to detergent formulations in which the ratio (based on weight) between anionic and nonionic surfactants is >1:1, preferably >1.5:1.

Thus, in a preferred embodiment the invention relates to a detergent composition wherein

(a) the endo-glucanase is selected from one of:
    (i) the endo-glucanase having the amino acid sequence of position 1 to position 773 of SEQ ID NO: 2;
    (ii) an endo-glucanase having a sequence of at least 90% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2; or a fragment thereof that has endo-glucanase activity; and
(b) the cellulase is selected from one of:
    (i) the cellulase having the amino acid sequence of position 1 to position 299 of SEQ ID NO: 4; or
    (ii) a cellulase having a sequence of at least 70% identity to the amino acid sequence of position 1 to position 299 of SEQ ID NO:4, or a fragment thereof that has cellulase activity.

Alternatively, the endo-glucanase of (a)(i) above is derived from *Bacillus* sp. KSMS237 deposited as FERM P-16067 (with the Patent Microorganism Depository, National Institute of Bioscience and Human-Technology, Agency of Industrial Science & Industry, Tsukuba-shi, Ibaraki, 305 Japan) and shown in position 1 to 824 of SEQ ID NO: 1 of JP 2000210081 A (hereby incorporated by reference).

Other alternative cellulases are the LAS resistant variants disclosed in Example 8 of WO 98/12307. Preferred embodiments of the invention comprise these alternative cellulases in combination with the endo-glucanases of (a) above.

In a preferred embodiment the cellulase is a *Thielavia terrestris* cellulase, preferably the cellulase disclosed in SEQ ID NO: 9 in WO 96/29397 and SEQ ID NO: 4 herein or an enzyme with at least 70% identity thereto. In a preferred embodiment the cellulase is the *Thielavia terrestris* variant disclosed in Example 1 of WO 98/12307.

<u>Identity</u>

In the context of the present invention the degree of identity is determined between two sequences indicating a derivation of the first sequence from the second. The identity is determined by means of the computer program GAP provided in the GCG program package. Thus, Gap GCGv8 is used with the following default parameters: GAP creation penalty of 3.0 and GAP

extension penalty of 0.1, the default scoring matrix, for protein sequences. GAP uses the method of Needleman/Wunsch/Sellers to make alignments.

Detergent compositions comprising other enzymes

Various types of enzymes are very commonly included in detergent formulations for laundry cleaning and for hard surface cleaning. One purpose of these enzymes is to degrade soil binding substances. These substances bind or attract soil and stains onto the fabric or hard surface. The degraded substances, and the associated soil and stain, are then more easily removed during the washing process. Redeposition of soil, which can be increased by soil binding substances, is prevented.

The substance on which an enzyme acts is called the substrate. Enzymes are known to be "substrate specific", i.e. each class of enzyme can only degrade one class of substances. For example, a protease can degrade proteins but cannot degrade starch. An amylase can degrade starch but cannot degrade proteins.

Because the soils and stains that are important for detergent formulators can contain many kinds of soil binding substances, a range of different enzymes, all with different substrate specificities have been developed for use in detergents. These include enzymes such as protease, amylase, beta-glucanase, lipase, hemi-cellulase, cutinase, pectinase and pectate lyase.

Surprisingly it has been found that the endo-glucanase of the invention are not substrate specific, in that they can provide detergency benefits on a wide range of soils and stains when used in combination with other enzymes.

The endo-glucanase of the present invention includes, in addition to the enzymes specified by reference to SEQ ID NO 2, other endo-glucanases with surprisingly high anti-redeposition effect. Two tests are provided in order to describe and identify these enzymes: 1) a test for endo-glucanase activity, and 2) a test for anti-redeposition effect. Enzymes which provide greater than the specified minimum performance on both these tests are regarded, for the purpose of this invention, as endo-glucanases having anti-redeposition effect.

Thus in a preferred embodiment an endo-glucanase of the invention is used together with an amylase to provide improved detergency performance on soils that contain starch. Such amylases comprise e.g. α- or β-amylases of bacterial or fungal origin. Chemically or genetically modified mutants of such amylases are included in this connection. Relevant α-amylases include, for example, α-amylases obtainable from *Bacillus* species, in particular a special strain of *B. licheniformis*, described in more detail in GB 1296839. Relevant commercially available amylases include Natalase™, Stainzyme®, Duramyl®, Termamyl®, Termamyl™ Ultra, Fungamyl® and BAN® (all available from Novozymes A/S, Bagsvaerd, Denmark), and Rapidase™ and Maxamyl™ P (available from DSM, Holland).

In a preferred embodiment the alpha-amylase is derived from *Bacillus* sp. strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 9375. Especially preferred are the alpha-amylases shown in SEQ ID NOS 1 and 2 of WO 95/26397.

In another preferred embodiment the alpha-amylase is the AA560 alpha-amylase derived from *Bacillus* sp. DSM 12649 disclosed as SEQ ID NO: 2 in WO 00/60060 (hereby incorporated by reference). Especially preferred are variants of the AA560 alpha-amylase, including the AA560 variant disclosed in Example 7 and 8 (hereby incorporated by reference).

Other useful amylases are CGTases (cyclodextrin glucanotransferases, EC 2.4.1.19), e.g. those obtainable from species of *Bacillus*, *Thermoanaerobactor* or *Thermoanaerobacterium*.

In another preferred embodiment an endo-glucanase of the invention is used together with a protease to provide improved detergency performance on soils that contain protein. Such proteases comprise those of animal, vegetable or microbial origin. Proteases of microbial origin are preferred. Chemically or genetically modified mutants of such proteases are included in this connection. The protease may be a serine protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270.

Relevant commercially available protease enzymes include Primase®, Durazym®, Everlase®, Kannase®, Alcalase®, Savinase® and Esperase® (all available from Novozymes A/S, Bagsvaerd, Denmark), Maxatase™, Maxacal™, Maxapem™ and Properase™ (available from DSM, Holland), Purafect™ and Purafect™ OXP (available from Genencor International, USA), and Opticlean™ and Optimase™ (available from by Solvay Enzymes).

In another preferred embodiment an endo-glucanase of the invention is used together with a lipase to provide improved detergency performance on soils that contain fat or oil. Such lipases comprise those of bacterial or fungal origin. Chemically or genetically modified mutants of such lipases are included in this connection.

Examples of useful lipases include a *Humicola lanuginosa* lipase, e.g. as described in EP 258 068 and EP 305 216; a *Rhizomucor miehei* lipase, e.g. as described in EP 238 023; a *Candida* lipase, such as a *C. antarctica* lipase, e.g. the *C. antarctica* lipase A or B described in EP 214 761; a *Pseudomonas lipase*, such as one of those described in EP 721 981 (e.g. a lipase obtainable from a *Pseudomonas* sp. SD705 strain having deposit accession number FERM BP-4772), in PCT/JP96/00426, in PCT/JP96/00454 (e.g. a *P. solanacearum* lipase), in EP 571 982 or in WO 95/14783 (e.g. a *P. mendocina* lipase), a *P. alcaligenes* or *P. pseudoalcaligenes* lipase, e.g. as described in EP 218 272, a *P. cepacia* lipase, e.g. as described in EP 331 376, a *P. stutzeri* lipase, e.g. as disclosed in GB 1,372,034, or a *P. fluorescens* lipase; a *Bacillus* lipase, e.g. a

e.g. a *B. subtilis* lipase (Dartois et al. (1993) Biochemica et Biophysica Acta 1131:253-260), a *B. stearothermophilus* lipase (JP 64/744992) and a *B. pumilus* lipase (WO 91/16422).

Other potentially useful types of lipolytic enzymes include cutinases, e.g. a cutinase derived from *Pseudomonas mendocina* as described in WO 88/09367, or a cutinase derived from *Fusarium solani* f. *pisi* (described, e.g., in WO 90/09446).

Suitable commercially available lipases include Lipex®, Lipolase® and Lipolase Ultra® (available from Novozymes A/S), M1 Lipase™ and Lipomax™ (available from Genencor Inc.) and Lipase P "Amano" (available from Amano Pharmaceutical Co. Ltd.). Commercially available cutinases include Lumafast™ from Genencor Inc.

In another preferred embodiment an endo-glucanase of the invention is used together with a hemi-cellulase to provide improved detergency performance on soils that contain hemi-cellulose and similar polysaccharides. Such hemi-cellulases include xylanases, xyloglucanases, arabinofuranosidases, acetyl xylan esterases, glucuronidases, ferulic acid esterases, coumaric acid esterases, endo-galactanases, mannanases, endo- or exo-arabinanases, exo-galactanases. Suitable mannanases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included.

In a preferred embodiment the mannanase is derived from a strain of the genus *Bacillus*, especially *Bacillus* sp. I633 disclosed in positions 31-330 of SEQ ID NO:2 or in SEQ ID NO: 5 of WO 99/64619 or *Bacillus agaradhaerens*, for example from the type strain DSM 8721. A suitable mannanase is Mannaway® produced by Novozymes A/S.

Thus in a preferred embodiment an endo-glucanase of the invention is used together with a beta-glucanase (EC 3.2.1.6) to provide improved detergency performance on soils that contain beta-glucans. Further preferred beta-glucanases include lichenases and laminarinases.

Thus in a preferred embodiment an endo-glucanase of the invention is used together with pectinolytic enzymes such as a protopectinase, pectinase, polygalacturonase or pectate lyase to provide improved detergency performance on pectinaceous soils. Suitable pectinolytic enzymes include those described in WO 99/27083, WO 99/27084, WO 00/55309 and WO 02/092741. Suitable pectate lyases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included.

In a preferred embodiment the pectate lyase is derived from a strain of the genus *Bacillus*, especially a strain of *Bacillus substilis*, especially *Bacillus subtilis* DSM14218 disclosed in SEQ ID NO:2 or a variant thereof disclosed in Example 6 of WO 02/092741.

Additionally, in a preferred embodiment an endo-glucanase of the invention is used together with other enzymes of the classes: pectate lyase (EC 4.2.2.2), pectin lyase (EC 4.2.2.10), rhamnogalacturonan lyase (EC not defined), endo-1,4-galactanase (EC 3.2.1.89), xyloglucanase (EC not defined), xylanase (EC 3.2.1.8), arabinanase (EC 3.2.1.99), alpha-L-

arabinofuranosidase (EC 3.2.1.55), Mannan endo-1,4-mannosidase (EC 3.2.1.78), beta-mannosidase (EC 3.2.1.25), beta-1,3-1,4-glucanase (EC 3.2.1.73), rhamnogalacturonan hydrolase, exo-polygalacturonase (EC 3.2.1.67), rhamnogalacturonase (EC not defined), Glucan 1,3-beta-glucosidase (EC 3.2.1.58), Glucan endo-1,6-beta-glucosidase (EC 3.2.1.75), Mannan endo-1,4-beta-mannosidase (EC 3.2.1.78), Endo-1,4-beta-xylanase (EC 3.2.1.8), Cellulose 1,4-cellobiosidase (EC 3.2.1.91), cellobiohydrolase (EC 3.2.1.91). Polygalacturonases (EC 3.2.1.15). Acetyl and methyl esterase enzymes such as: rhamnogalacturonan methyl esterase, rhamnogalacturonan acetyl esterase, pectin methylesterase (EC 3.1.1.11), pectin acetylesterase (EC not defined), xylan methyl esterase, acetyl xylan esterase (EC 3.1.1.72), feruloyl esterase (EC 3.1.1.73), cinnamoyl esterase (EC 3.1.1.73) to provide improved detergency performance on corresponding soils.

Detergent composition of the invention

The detergent compositions according to the present invention comprise a surfactant system, wherein the surfactant can be selected from nonionic and/or anionic and/or cationic and/or amphoteric and/or zwitterionic and/or semi-polar surfactants.

The surfactant is typically present at a level from 0.1% to 60% by weight.

The surfactant is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated in such a way that it promotes, or at least does not degrade, the stability of any enzyme in these compositions.

Preferred systems to be used according to the present invention comprise as a surfactant one or more of the nonionic and/or anionic surfactants described herein.

Polyethylene, polypropylene, and polybutylene oxide condensates of alkyl phenols are suitable for use as the nonionic surfactant of the surfactant systems of the present invention, with the polyethylene oxide condensates being preferred. These compounds include the condensation products of alkyl phenols having an alkyl group containing from about 6 to about 14 carbon atoms, preferably from about 8 to about 14 carbon atoms, in either a straight chain or branched-chain configuration with the alkylene oxide. In a preferred embodiment, the ethylene oxide is present in an amount equal to from about 2 to about 25 moles, more preferably from about 3 to about 15 moles, of ethylene oxide per mole of alkyl phenol. Commercially available nonionic surfactants of this type include Igepal™ CO-630, marketed by the GAF Corporation; and Triton™ X-45, X-114, X-100 and X-102, all marketed by the Rohm & Haas Company. These surfactants are commonly referred to as alkylphenol alkoxylates (e.g., alkyl phenol ethoxylates).

The condensation products of primary and secondary aliphatic alcohols with about 1 to about 25 moles of ethylene oxide are suitable for use as the nonionic surfactant of the

nonionic surfactant systems of the present invention. The alkyl chain of the aliphatic alcohol can either be straight or branched, primary or secondary, and generally contains from about 8 to about 22 carbon atoms. Preferred are the condensation products of alcohols having an alkyl group containing from about 8 to about 20 carbon atoms, more preferably from about 10 to about 18 carbon atoms, with from about 2 to about 10 moles of ethylene oxide per mole of alcohol. About 2 to about 7 moles of ethylene oxide and most preferably from 2 to 5 moles of ethylene oxide per mole of alcohol are present in said condensation products. Examples of commercially available nonionic surfactants of this type include Tergitol™ 15-S-9 (The condensation product of C_{11}-C_{15} linear alcohol with 9 moles ethylene oxide), Tergitol™ 24-L-6 NMW (the condensation product of C_{12}-C_{14} primary alcohol with 6 moles ethylene oxide with a narrow molecular weight distribution), both marketed by Union Carbide Corporation; Neodol™ 45-9 (the condensation product of C_{14}-C_{15} linear alcohol with 9 moles of ethylene oxide), Neodol™ 23-3 (the condensation product of C_{12}-C_{13} linear alcohol with 3.0 moles of ethylene oxide), Neodol™ 45-7 (the condensation product of C_{14}-C_{15} linear alcohol with 7 moles of ethylene oxide), Neodol™ 45-5 (the condensation product of C_{14}-C_{15} linear alcohol with 5 moles of ethylene oxide) marketed by Shell Chemical Company, Kyro™ EOB (the condensation product of C_{13}-C_{15} alcohol with 9 moles ethylene oxide), marketed by The Procter & Gamble Company, and Genapol LA 050 (the condensation product of C_{12}-C_{14} alcohol with 5 moles of ethylene oxide) marketed by Hoechst. Preferred range of HLB in these products is from 8-11 and most preferred from 8-10.

Also useful as the nonionic surfactant of the surfactant systems of the present invention are alkylpolysaccharides disclosed in U S 4,565,647, having a hydrophobic group containing from about 6 to about 30 carbon atoms, preferably from about 10 to about 16 carbon atoms and a polysaccharide, e.g. a polyglycoside, hydrophilic group containing from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7 saccharide units. Any reducing saccharide containing 5 or 6 carbon atoms can be used, e.g., glucose, galactose and galactosyl moieties can be substituted for the glucosyl moieties (optionally the hydrophobic group is attached at the 2-, 3-, 4-, etc. positions thus giving a glucose or galactose as opposed to a glucoside or galactoside). The intersaccharide bonds can be, e.g., between the one position of the additional saccharide units and the 2-, 3-, 4-, and/or 6- positions on the preceding saccharide units.

The preferred alkylpolyglycosides have the formula

$$R^2O(C_nH_{2n}O)_t(\text{glycosyl})_x$$

wherein R^2 is selected from the group consisting of alkyl, alkylphenyl, hydroxyalkyl, hydroxyalkylphenyl, and mixtures thereof in which the alkyl groups contain from about 10 to

about 18, preferably from about 12 to about 14, carbon atoms; n is 2 or 3, preferably 2; t is from 0 to about 10, preferably 0; and x is from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7. The glycosyl is preferably derived from glucose. To prepare these compounds, the alcohol or alkylpolyethoxy alcohol is formed first and then reacted with glucose, or a source of glucose, to form the glucoside (attachment at the 1-position). The additional glycosyl units can then be attached between their 1-position and the preceding glycosyl units 2-, 3-, 4-, and/or 6-position, preferably predominantly the 2-position.

The condensation products of ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol are also suitable for use as the additional nonionic surfactant systems of the present invention. The hydrophobic portion of these compounds will preferably have a molecular weight from about 1500 to about 1800 and will exhibit water insolubility. The addition of polyoxyethylene moieties to this hydrophobic portion tends to increase the water solubility of the molecule as a whole, and the liquid character of the product is retained up to the point where the polyoxyethylene content is about 50% of the total weight of the condensation product, which corresponds to condensation with up to about 40 moles of ethylene oxide. Examples of compounds of this type include certain of the commercially available Pluronic™ surfactants, marketed by BASF.

Also suitable for use as the nonionic surfactant of the nonionic surfactant system of the present invention, are the condensation products of ethylene oxide with the product resulting from the reaction of propylene oxide and ethylenediamine. The hydrophobic moiety of these products consists of the reaction product of ethylenediamine and excess propylene oxide, and generally has a molecular weight of from about 2500 to about 3000. This hydrophobic moiety is condensed with ethylene oxide to the extent that the condensation product contains from about 40% to about 80% by weight of polyoxyethylene and has a molecular weight of from about 5,000 to about 11,000. Examples of this type of nonionic surfactant include certain of the commercially available Tetronic™ compounds, marketed by BASF.

Preferred for use as the nonionic surfactant of the surfactant systems of the present invention are polyethylene oxide condensates of alkyl phenols, condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethyleneoxide, alkylpolysaccharides, and mixtures hereof. Most preferred are C_8-C_{14} alkyl phenol ethoxylates having from 3 to 15 ethoxy groups and C_8-C_{18} alcohol ethoxylates (preferably C_{10} avg.) having from 2 to 10 ethoxy groups, and mixtures thereof.

Highly preferred nonionic surfactants are polyhydroxy fatty acid amide surfactants of the formula

$$R^2 - C - N - Z,$$

$$\begin{array}{cc} \| & \| \\ O & R^{1} \end{array}$$

wherein R^1 is H, or R^1 is C_{1-4} hydrocarbyl, 2-hydroxyethyl, 2-hydroxypropyl or a mixture thereof, R^2 is C_{5-31} hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R^1 is methyl, R^2 is straight C_{11-15} alkyl or C_{16-18} alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose or lactose, in a reductive amination reaction.

Highly preferred anionic surfactants include alkyl alkoxylated sulfate surfactants. Examples hereof are water soluble salts or acids of the formula $RO(A)_mSO_3M$ wherein R is an unsubstituted C_{10}-C-$_{24}$ alkyl or hydroxyalkyl group having a C_{10}-C_{24} alkyl component, preferably a C_{12}-C_{20} alkyl or hydro-xyalkyl, more preferably C_{12}-C_{18} alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl, trimethyl-ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and those derived from alkylamines such as ethylamine, diethylamine, triethylamine, mixtures thereof, and the like. Exemplary surfactants are C_{12}-C_{18} alkyl polyethoxylate (1.0) sulfate (C_{12}-$C_{18}E(1.0)M$), C_{12}-C_{18} alkyl polyethoxylate (2.25) sulfate (C_{12}-$C_{18}(2.25)M$, and C_{12}-C_{18} alkyl polyethoxylate (3.0) sulfate (C_{12}-$C_{18}E(3.0)M$), and C_{12}-C_{18} alkyl polyethoxylate (4.0) sulfate (C_{12}-$C_{18}E(4.0)M$), wherein M is conveniently selected from sodium and potassium.

Suitable anionic surfactants to be used are alkyl ester sulfonate surfactants including linear esters of C_8-C_{20} carboxylic acids (i.e., fatty acids) which are sulfonated with gaseous SO_3 according to "The Journal of the American Oil Chemists Society", 52 (1975), pp. 323-329. Suitable starting materials would include natural fatty substances as derived from tallow, palm oil, etc.

The preferred alkyl ester sulfonate surfactant, especially for laundry applications, comprises alkyl ester sulfonate surfactants of the structural formula:

$$R^3-CH-\underset{\underset{SO_3M}{|}}{\overset{\overset{O}{\|}}{C}}-OR^4$$

wherein R^3 is a C_8-C_{20} hydrocarbyl, preferably an alkyl, or combination thereof, R^4 is a C_1-C_6 hydrocarbyl, preferably an alkyl, or combination thereof, and M is a cation which forms a water soluble salt with the alkyl ester sulfonate. Suitable salt-forming cations include metals such as sodium, potassium, and lithium, and substituted or unsubstituted ammonium cations, such as monoethanolamine, diethonolamine, and triethanolamine. Preferably, R^3 is C_{10}-C_{16} alkyl, and R^4 is methyl, ethyl or isopropyl. Especially preferred are the methyl ester sulfonates wherein R^3 is C_{10}-C_{16} alkyl.

Other suitable anionic surfactants include the alkyl sulfate surfactants which are water soluble salts or acids of the formula $ROSO_3M$ wherein R preferably is a C_{10}-C_{24} hydrocarbyl, preferably an alkyl or hydroxyalkyl having a C_{10}-C_{20} alkyl component, more preferably a C_{12}-C_{18} alkyl or hydroxyalkyl, and M is H or a cation, e.g., an alkali metal cation (e.g. sodium, potassium, lithium), or ammonium or substituted ammonium (e.g. methyl-, dimethyl-, and trimethyl ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and quaternary ammonium cations derived from alkylamines such as ethylamine, diethylamine, triethylamine, and mixtures thereof, and the like). Typically, alkyl chains of C_{12}-C_{16} are preferred for lower wash temperatures (e.g. below about 50°C) and C_{16}-C_{18} alkyl chains are preferred for higher wash temperatures (e.g. above about 50°C).

Other anionic surfactants useful for detersive purposes can also be included in the laundry detergent compositions of the present invention. Theses can include salts (including, for example, sodium, potassium, ammonium, and substituted ammonium salts such as mono- di- and triethanolamine salts) of soap, C_8-C_{22} primary or secondary alkanesulfonates, C_8-C_{24} olefinsulfonates, sulfonated polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkaline earth metal citrates, e.g., as described in British patent specification No. 1,082,179, C_8-C_{24} alkylpolyglycolethersulfates (containing up to 10 moles of ethylene oxide); alkyl glycerol sulfonates, fatty acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates such as the acyl isethionates, N-acyl taurates, alkyl succinamates and sulfosuccinates, monoesters of sulfosuccinates (especially saturated and unsaturated C_{12}-C_{18} monoesters) and diesters of sulfosuccinates (especially saturated and unsaturated C_6-C_{12} diesters), acyl sarcosinates, sulfates of alkylpolysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, and alkyl polyethoxy carboxylates such as those of the formula $RO(CH_2CH_2O)_k$-CH_2C00-M+ wherein R is a C_8-C_{22} alkyl, k is an integer from 1 to 10, and M is a soluble salt forming cation. Resin acids and hydrogenated resin acids are also suitable, such as rosin, hydrogenated rosin, and resin acids and hydrogenated resin acids present in or derived from tall oil.

Alkylbenzene sulfonates are highly preferred. Especially preferred are linear (straight-chain) alkyl benzene sulfonates (LAS) wherein the alkyl group preferably contains from 10 to 18 carbon atoms.

Further examples are described in "Surface Active Agents and Detergents" (Vol. I and II by Schwartz, Perrry and Berch). A variety of such surfactants are also generally disclosed in US 3,929,678, (Column 23, line 58 through Column 29, line 23, herein incorporated by reference).

When included therein, the detergent compositions of the present invention typically comprise from about 1% to about 40%, preferably from about 3% to about 25 % by weight of such anionic surfactants.

The laundry detergent compositions of the present invention may also contain cationic, amphoteric, zwitterionic, and semi-polar surfactants, as well as the nonionic and/or anionic surfactants other than those already described herein.

Cationic detersive surfactants suitable for use in the laundry detergent compositions of the present invention are those having one long-chain hydrocarbyl group. Examples of such cationic surfactants include the ammonium surfactants such as alkyltrimethylammonium halogenides, and those surfactants having the formula:

$$[R^2(OR^3)_y][R^4(OR^3)_y]_2R^5N^+X^-$$

wherein R^2 is an alkyl or alkyl benzyl group having from about 8 to about 18 carbon atoms in the alkyl chain, each R^3 is selected form the group consisting of $-CH_2CH_2-$, $-CH_2CH(CH_3)-$, $-CH_2CH(CH_2OH)-$, $-CH_2CH_2CH_2-$, and mixtures thereof; each R^4 is selected from the group consisting of C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, benzyl ring structures formed by joining the two R^4 groups, $-CH_2CHOHCHOHCOR^6CHOHCH_2OH$, wherein R^6 is any hexose or hexose polymer having a molecular weight less than about 1000, and hydrogen when y is not 0; R^5 is the same as R^4 or is an alkyl chain, wherein the total number of carbon atoms of R^2 plus R^5 is not more than about 18; each y is from 0 to about 10,and the sum of the y values is from 0 to about 15; and X is any compatible anion.

Highly preferred cationic surfactants are the water soluble quaternary ammonium compounds useful in the present composition having the formula:

$$R_1R_2R_3R_4N^+X^- \quad (\text{i})$$

wherein R_1 is C_8-C_{16} alkyl, each of R_2, R_3 and R_4 is independently C_1-C_4 alkyl, C_1-C_4 hydroxy alkyl, benzyl, and $-(C_2H_{40})_xH$ where x has a value from 2 to 5, and X is an anion. Not more than one of R_2, R_3 or R_4 should be benzyl.

The preferred alkyl chain length for R_1 is C_{12}-C_{15}, particularly where the alkyl group is a mixture of chain lengths derived from coconut or palm kernel fat or is derived synthetically by olefin build up or OXO alcohols synthesis.

Preferred groups for R_2, R_3 and R_4 are methyl and hydroxyethyl groups and the anion X may be selected from halide, methosulphate, acetate and phosphate ions.

Examples of suitable quaternary ammonium compounds of formulae (i) for use herein are:

coconut trimethyl ammonium chloride or bromide;
coconut methyl dihydroxyethyl ammonium chloride or bromide;
decyl triethyl ammonium chloride;
decyl dimethyl hydroxyethyl ammonium chloride or bromide;
$C_{12\text{-}15}$ dimethyl hydroxyethyl ammonium chloride or bromide;
coconut dimethyl hydroxyethyl ammonium chloride or bromide;
myristyl trimethyl ammonium methyl sulphate;
lauryl dimethyl benzyl ammonium chloride or bromide;
lauryl dimethyl (ethenoxy)$_4$ ammonium chloride or bromide;
choline esters (compounds of formula (i) wherein R_1 is

$$CH_2\text{-}CH_2\text{-}O\text{-}\underset{\underset{\displaystyle O}{||}}{C}\text{-}C_{12\text{-}14} \text{ alkyl and } R_2R_3R_4 \text{ are methyl).}$$

di-alkyl imidazolines [compounds of formula (i)].

Other cationic surfactants useful herein are also described in US 4,228,044 and in EP 000 224.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 25%, preferably from about 1% to about 8% by weight of such cationic surfactants.

Amphoteric surfactants are also suitable for use in the detergent compositions of the present invention. These surfactants can be broadly described as aliphatic derivatives of secondary or tertiary amines, or aliphatic derivatives of heterocyclic secondary and tertiary amines in which the aliphatic radical can be straight- or branched-chain. One of the aliphatic substituents contains at least about 8 carbon atoms, typically from about 8 to about 18 carbon atoms, and at least one contains an anionic water-solubilizing group, e.g. carboxy, sulfonate, sulfate. See US 3,929,678 (column 19, lines 18-35) for examples of amphoteric surfactants.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such amphoteric surfactants.

Zwitterionic surfactants are also suitable for use in detergent compositions. These surfactants can be broadly described as derivatives of secondary and tertiary amines, derivatives of heterocyclic secondary and tertiary amines, or derivatives of quaternary ammonium, quaternary phosphonium or tertiary sulfonium compounds. See US 3,929,678 (column 19, line 38 through column 22, line 48) for examples of zwitterionic surfactants.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such zwitterionic surfactants.

Semi-polar nonionic surfactants are a special category of nonionic surfactants which include water-soluble amine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; water soluble phosphine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms.

Semi-polar nonionic detergent surfactants include the amine oxide surfactants having the formula:

$$\overset{\displaystyle O}{\uparrow}$$
$$R^3(OR^4)xN(R^5)2$$

wherein R^3 is an alkyl, hydroxyalkyl, or alkyl phenyl group or mixtures thereof containing from about 8 to about 22 carbon atoms; R^4 is an alkylene or hydroxyalkylene group containing from about 2 to about 3 carbon atoms or mixtures thereof; x is from 0 to about 3: and each R^5 is an alkyl or hydroxyalkyl group containing from about 1 to about 3 carbon atoms or a polyethylene oxide group containing from about 1 to about 3 ethylene oxide groups. The R^5 groups can be attached to each other, e.g., through an oxygen or nitrogen atom, to form a ring structure.

These amine oxide surfactants in particular include C_{10}-C_{18} alkyl dimethyl amine oxides and C_8-C_{12} alkoxy ethyl dihydroxy ethyl amine oxides.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such semi-polar nonionic surfactants.

Builder system

The compositions according to the present invention may further comprise a builder system. Any conventional builder system is suitable for use herein including aluminosilicate

materials, silicates, polycarboxylates and fatty acids, materials such as ethylenediamine tetraacetate, metal ion sequestrants such as aminopolyphosphonates, particularly ethylenediamine tetramethylene phosphonic acid and diethylene triamine pentamethylenephosphonic acid. Though less preferred for obvious environmental reasons, phosphate builders can also be used herein.

Suitable builders can be an inorganic ion exchange material, commonly an inorganic hydrated aluminosilicate material, more particularly a hydrated synthetic zeolite such as hydrated zeolite A, X, B, HS or MAP.

Another suitable inorganic builder material is layered silicate, e.g. SKS-6 (Hoechst). SKS-6 is a crystalline layered silicate consisting of sodium silicate ($Na_2Si_2O_5$).

Suitable polycarboxylates containing one carboxy group include lactic acid, glycolic acid and ether derivatives thereof as disclosed in Belgian Patent Nos. 831, 368, 821,369 and 821,370. Polycarboxylates containing two carboxy groups include the water-soluble salts of succinic acid, malonic acid, (ethylenedioxy) diacetic acid, maleic acid, diglycollic acid, tartaric acid, tartronic acid and fumaric acid, as well as the ether carboxylates described in DE 2,446,686, and 2,446,487, US 3,935,257 and the sulfinyl carboxylates described in Belgian Patent No. 840,623. Polycarboxylates containing three carboxy groups include, in particular, water-soluble citrates, aconitrates and citraconates as well as succinate derivatives such as the carboxymethyloxysuccinates described in British Patent No. 1,379,241, lactoxysuccinates described in Netherlands Application 7205873, and the oxypolycarboxylate materials such as 2-oxa-1,1,3-propane tricarboxylates described in British Patent No. 1,387,447.

Polycarboxylates containing four carboxy groups include oxydisuccinates disclosed in British Patent No. 1,261,829, 1,1,2,2,-ethane tetracarboxylates, 1,1,3,3-propane tetracarboxylates containing sulfo substituents include the sulfosuccinate derivatives disclosed in British Patent Nos. 1,398,421 and 1,398,422 and in US 3,936,448, and the sulfonated pyrolysed citrates described in British Patent No. 1,082,179, while polycarboxylates containing phosphone substituents are disclosed in British Patent No. 1,439,000.

Alicyclic and heterocyclic polycarboxylates include cyclopentane-cis,cis-cis-tetracarboxylates, cyclopentadienide pentacarboxylates, 2,3,4,5-tetrahydro-furan - cis, cis, cis-tetracarboxylates, 2,5-tetrahydro-furan-cis, discarboxylates, 2,2,5,5,-tetrahydrofuran - tetracarboxylates, 1,2,3,4,5,6-hexane - hexacarboxylates and carboxymethyl derivatives of polyhydric alcohols such as sorbitol, mannitol and xylitol. Aromatic polycarboxylates include mellitic acid, pyromellitic acid and the phthalic acid derivatives disclosed in British Patent No. 1,425,343.

Of the above, the preferred polycarboxylates are hydroxy-carboxylates containing up to three carboxy groups per molecule, more particularly citrates.

Preferred builder systems for use in the present compositions include a mixture of a water-insoluble aluminosilicate builder such as zeolite A or of a layered silicate (SKS-6), and a water-soluble carboxylate chelating agent such as citric acid.

A suitable chelant for inclusion in the detergent compositions in accordance with the invention is ethylenediamine-N,N'-disuccinic acid (EDDS) or the alkali metal, alkaline earth metal, ammonium, or substituted ammonium salts thereof, or mixtures thereof. Preferred EDDS compounds are the free acid form and the sodium or magnesium salt thereof. Examples of such preferred sodium salts of EDDS include Na_2EDDS and Na_4EDDS. Examples of such preferred magnesium salts of EDDS include MgEDDS and Mg_2EDDS. The magnesium salts are the most preferred for inclusion in compositions in accordance with the invention.

Other builder materials that can form part of the builder system for use in granular compositions include inorganic materials such as alkali metal carbonates, bicarbonates, silicates, and organic materials such as the organic phosphonates, amino polyalkylene phosphonates and amino polycarboxylates.

Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated form each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

Detergency builder salts are normally included in amounts of from 5% to 80% by weight of the composition. Preferred levels of builder for liquid detergents are from 5% to 30%.

Bleaching agents: Additional optional detergent ingredients that can be included in the detergent compositions of the present invention include bleaching agents such as perborate PB1, PB4 and percarbonate. These bleaching agent components can include one or more oxygen bleaching agents and, depending upon the bleaching agent chosen, one or more bleach activators. Present oxygen bleaching compounds will typically be present at levels of from about 1% to about 25%. In general, bleaching compounds are optional added components in non-liquid formulations, e.g. granular detergents.

The bleaching agent component for use herein can be any of the bleaching agents useful for detergent compositions including oxygen bleaches as well as others known in the art.

The bleaching agent suitable for the present invention can be an activated or non-activated bleaching agent.

One category of oxygen bleaching agent that can be used encompasses percarboxylic acid bleaching agents and salts thereof. Suitable examples of this class of agents include magnesium monoperoxyphthalate hexahydrate, the magnesium salt of meta-chloro perbenzoic acid, 4-nonylamino-4-oxoperoxybutyric acid and diperoxydodecanedioic acid. Such bleaching agents are disclosed in US 4,483,781, EP 0 133 354 and US 4,412,934. Highly preferred bleaching agents also include 6-nonylamino-6-oxoperoxycaproic acid as described in US 4,634,551.

Another category of bleaching agents encompasses the halogen bleaching agents. Examples of hypohalite bleaching agents, for example, include trichloro isocyanuric acid and the sodium and potassium dichloroisocyanurates and N-chloro and N-bromo alkane sulphonamides. Such materials are normally added at 0.5-10% by weight of the finished product, preferably 1-5% by weight. Such halogen bleaching agents are generally less preferred for use in enzymatic detergents.

The hydrogen peroxide releasing agents can be used in combination with bleach activators such as tetra-acetylethylenediamine (TAED), nonanoyloxybenzenesulfonate (NOBS, described in US 4,412,934), 3,5-trimethyl-hexsanoloxybenzenesulfonate (ISONOBS, described in EP 120 591) or pentaacetylglucose (PAG), which are perhydrolyzed to form a peracid as the active bleaching species, leading to improved bleaching effect. In addition, very suitable are the bleach activators C8 (6-octanamido-caproyl) oxybenzene-sulfonate, C9(6-nonanamido caproyl) oxybenzenesulfonate and C10 (6-decanamido caproyl) oxybenzene-sulfonate or mixtures thereof. Also suitable activators are acylated citrate esters such as disclosed in European Patent Application No. 91870207.7.

Useful bleaching agents, including peroxyacids and bleaching systems comprising bleach activators and peroxygen bleaching compounds for use in cleaning compositions according to the invention are described in application USSN 08/136,626.

The hydrogen peroxide may also be present by adding an enzymatic system (i.e. an enzyme and a substrate therefore) which is capable of generation of hydrogen peroxide at the beginning or during the washing and/or rinsing process. Such enzymatic systems are disclosed in European Patent Application EP 0 537 381.

Bleaching agents other than oxygen bleaching agents are also known in the art and can be utilized herein. One type of non-oxygen bleaching agent of particular interest includes photoactivated bleaching agents such as the sulfonated zinc and/or aluminium phthalocyanines. These materials can be deposited upon the substrate during the washing process. Upon irradiation with light, in the presence of oxygen, such as by hanging clothes out to dry in the daylight, the sulfonated zinc phthalocyanine is activated and, consequently, the substrate is bleached. Preferred zinc phthalocyanine and a photoactivated bleaching process

are described in US 4,033,718. Typically, detergent composition will contain about 0.025% to about 1.25%, by weight, of sulfonated zinc phthalocyanine.

Bleaching agents may also comprise a manganese catalyst. The manganese catalyst may, e.g., be one of the compounds described in "Efficient manganese catalysts for low-temperature bleaching", Nature (369) 1994, pp. 637-639.

Suds suppressors: Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Silicones can generally be represented by alkylated polysiloxane materials, while silica is normally used in finely divided forms exemplified by silica aerogels and xerogels and hydrophobic silicas of various types. Theses materials can be incorporated as particulates, in which the suds suppressor is advantageously releasably incorporated in a water-soluble or water-dispersible, substantially non surface-active detergent impermeable carrier. Alternatively the suds suppressor can be dissolved or dispersed in a liquid carrier and applied by spraying onto one or more of the other components.

A preferred silicone suds controlling agent is disclosed in US 3,933,672. Other particularly useful suds suppressors are the self-emulsifying silicone suds suppressors, described in German Patent Application DTOS 2,646,126. An example of such a compound is DC-544, commercially available form Dow Corning, which is a siloxane-glycol copolymer. Especially preferred suds controlling agent are the suds suppressor system comprising a mixture of silicone oils and 2-alkyl-alkanols. Suitable 2-alkyl-alkanols are 2-butyl-octanols, which are commercially available under the trade name Isofol 12 R.

Such suds suppressor system are described in European Patent Application EP 0 593 841.

Especially preferred silicone suds controlling agents are described in European Patent Application No. 92201649.8. Said compositions can comprise a silicone/ silica mixture in combination with fumed nonporous silica such as Aerosil®.

The suds suppressors described above are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight.

Other components: Other components used in detergent compositions may be employed such as soil-suspending or anti-redeposition agents, soil-releasing agents, optical brighteners, abrasives, bactericides, tarnish inhibitors, coloring agents, and/or encapsulated or non-encapsulated perfumes.

Especially suitable encapsulating materials are water soluble capsules which consist of a matrix of polysaccharide and polyhydroxy compounds such as described in GB 1,464,616.

Other suitable water soluble encapsulating materials comprise dextrins derived from ungelatinized starch acid esters of substituted dicarboxylic acids such as described in US 3,455,838. These acid-ester dextrins are, preferably, prepared from such starches as waxy maize, waxy sorghum, sago, tapioca and potato. Suitable examples of said encapsulation materials include N-Lok manufactured by National Starch. The N-Lok encapsulating material consists of a modified maize starch and glucose. The starch is modified by adding monofunctional substituted groups such as octenyl succinic acid anhydride.

Typical anti-redeposition agents used in detergents include water-soluble, generally organic colloids, including for example the water-soluble salts of polymeric carboxylic acids such as polyacrylic acid or polymaleic acid or co-polymers thereof, glue, gelatine, salts of ether carboxylic acids or ether sulfonic acids of starch or cellulose or salts of sulfuric acid esters of cellulose or starch. Water-soluble polyamides containing acidic groups are also used as anti-redeposition agent. Soluble starch preparations and other starch products than those mentioned above, for example partly hydrolyzed starch, may also be used. Sodium carboxymethyl cellulose, methyl cellulose, hydroxyethyl cellulose, methyl hydroxyethyl cellulose and mixtures thereof are preferably used. These materials are normally used at levels of from 0.05% to 10% by weight, more preferably form 0.2% to 8%, most preferably from 0.5% to 6% by weight of the composition.

Preferred optical brighteners are anionic in character, examples of which are disodium 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-ylamino)stilbene-2:2' disulpho-nate, disodium 4, - 4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino-stilbene-2:2' - disulphonate, disodium 4,4' - bis-(2,4-dianilino-s-triazin-6-ylamino)stilbene-2:2' - disulpho-nate, monosodium 4',4" - bis-(2,4-dianilino-s-tri-azin-6-ylamino)stilbene-2-sulphonate, disodium 4,4'-bis-(2-anilino-4-(N-methyl-N-2-hydroxyethylamino)-s-triazin-6-ylamino) stilbene-2,2' - disulphonate, di-sodium 4,4' -bis-(4-phenyl-2,1,3-triazol-2-yl)-stilbene-2,2' disulphonate, disodium 4,4'bis(2-anilino-4-(1-methyl-2-hydroxyethylamino)-s-triazin-6-ylami-no)stilbene-2,2'disulphonate, sodium 2(stilbyl-4"-(naphtho-1',2':4,5)-1,2,3, - triazole-2"-sulphonate and 4,4'-bis(2-sulphostyryl)biphenyl.

Other useful polymeric materials are the polyethylene glycols, particularly those of molecular weight 1000-10000, more particularly 2000 to 8000 and most preferably about 4000. These are used at levels of from 0.20% to 5% more preferably from 0.25% to 2.5% by weight. These polymers and the previously mentioned homo- or co-polymeric poly-carboxylate salts are valuable for improving whiteness maintenance, fabric ash deposition, and cleaning performance on clay, proteinaceous and oxidizable soils in the presence of transition metal impurities.

Soil release agents useful in compositions of the present invention are conventionally copolymers or terpolymers of terephthalic acid with ethylene glycol and/or propylene glycol

units in various arrangements. Examples of such polymers are disclosed in US 4,116,885 and 4,711,730 and EP 0 272 033. A particular preferred polymer in accordance with EP 0 272 033 has the formula:

$$(CH_3(PEG)_{43})_{0.75}(POH)_{0.25}[T\text{-}PO)_{2.8}(T\text{-}PEG)_{0.4}]T(POH)_{0.25}((PEG)_{43}CH_3)_{0.75}$$

where PEG is -(OC_2H_4)0-, PO is (OC_3H_6O) and T is ($pOOC_6H_4CO$).

Also very useful are modified polyesters as random copolymers of dimethyl terephthalate, dimethyl sulfoisophthalate, ethylene glycol and 1,2-propanediol, the end groups consisting primarily of sulphobenzoate and secondarily of mono esters of ethylene glycol and/or 1,2-propanediol. The target is to obtain a polymer capped at both end by sulphobenzoate groups, "primarily", in the present context most of said copolymers herein will be endcapped by sulphobenzoate groups. However, some copolymers will be less than fully capped, and therefore their end groups may consist of monoester of ethylene glycol and/or 1,2-propanediol, thereof consist "secondarily" of such species.

The selected polyesters herein contain about 46% by weight of dimethyl terephthalic acid, about 16% by weight of 1,2-propanediol, about 10% by weight ethylene glycol, about 13% by weight of dimethyl sulfobenzoic acid and about 15% by weight of sulfoisophthalic acid, and have a molecular weight of about 3.000. The polyesters and their method of preparation are described in detail in EP 311 342.

Softening agents: Fabric softening agents can also be incorporated into laundry detergent compositions in accordance with the present invention. These agents may be inorganic or organic in type. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1 400898 and in US 5,019,292. Organic fabric softening agents include the water insoluble tertiary amines as disclosed in GB-A1 514 276 and EP 0 011 340 and their combination with mono C_{12}-C_{14} quaternary ammonium salts are disclosed in EP-B-0 026 528 and di-long-chain amides as disclosed in EP 0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP 0 299 575 and 0 313 146.

Levels of smectite clay are normally in the range from 5% to 15%, more preferably from 8% to 12% by weight, with the material being added as a dry mixed component to the remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or di-long chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight. These materials are normally added to the spray

dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as molten liquid on to other solid components of the composition.

Polymeric dye-transfer inhibiting agents: The detergent compositions according to the present invention may also comprise from 0.001% to 10%, preferably from 0.01% to 2%, more preferably form 0.05% to 1% by weight of polymeric dye- transfer inhibiting agents. Said polymeric dye-transfer inhibiting agents are normally incorporated into detergent compositions in order to inhibit the transfer of dyes from colored fabrics onto fabrics washed therewith. These polymers have the ability of complexing or adsorbing the fugitive dyes washed out of dyed fabrics before the dyes have the opportunity to become attached to other articles in the wash.

Especially suitable polymeric dye-transfer inhibiting agents are polyamine N-oxide polymers, copolymers of N-vinyl-pyrrolidone and N-vinylimidazole, polyvinylpyrrolidone polymers, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof.

Addition of such polymers also enhances the performance of the enzymes according the invention.

Enzymes

A detergent composition of the invention may in an embodiment of the invention besides the endo-glucanase having anti-redeposition effect as defined above, comprise other enzyme(s) which provides cleaning performance and/or fabric care benefits.

Such enzymes include certain proteases, lipases, cutinases, cellulases, amylases, peroxidases, oxidases (e.g. laccases), and hemicellulases such as mannanase and pectate lyase.

Proteases: Any protease suitable for use in alkaline solutions can be used. Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically or genetically modified mutants are included. The protease may be a serine protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270.

Preferred commercially available protease enzymes include those sold under the trade names Everlase™, Kannase™, Alcalase™, Savinase™, Primase™, Durazym™, and Esperase™ by Novozymes A/S (Denmark), those sold under the tradename Maxatase,

Maxacal, Maxapem, Properase, Purafect and Purafect OXP by Genencor International, and those sold under the tradename Opticlean and Optimase by Solvay Enzymes. Protease enzymes may be incorporated into the compositions in accordance with the invention at a level of from 0.000001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.00001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.0001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.001% to 0.2% of enzyme protein by weight of the composition.

Lipases: Any lipase suitable for use in alkaline solutions can be used. Suitable lipases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included.

Examples of useful lipases include a *Humicola lanuginosa* lipase, e.g., as described in EP 258 068 and EP 305 216, a *Rhizomucor miehei* lipase, e.g., as described in EP 238 023, a *Candida* lipase, such as a *C. antarctica* lipase, e.g., the *C. antarctica* lipase A or B described in EP 214 761, a *Pseudomonas* lipase such as a *P. alcaligenes* and *P. pseudoalcaligenes* lipase, e.g., as described in EP 218 272, a *P. cepacia* lipase, e.g., as described in EP 331 376, a *P. stutzeri* lipase, e.g., as disclosed in GB 1,372,034, a *P. fluorescens* lipase, a *Bacillus* lipase, e.g., a *B. subtilis* lipase (Dartois et al., (1993), Biochemica et Biophysica acta 1131, 253-260), a *B. stearothermophilus* lipase (JP 64/744992) and a *B. pumilus* lipase (WO 91/16422).

Furthermore, a number of cloned lipases may be useful, including the *Penicillium camembertii* lipase described by Yamaguchi et al., (1991), Gene 103, 61-67), the *Geotricum candidum* lipase (Schimada, Y. et al., (1989), J. Biochem., 106, 383-388), and various *Rhizopus* lipases such as a *R. delemar* lipase (Hass, M.J et al., (1991), Gene 109, 117-113), a *R. niveus* lipase (Kugimiya et al., (1992), Biosci. Biotech. Biochem. 56, 716-719) and a *R. oryzae* lipase.

Other types of lipolytic enzymes such as cutinases may also be useful, e.g., a cutinase derived from *Pseudomonas mendocina* as described in WO 88/09367, or a cutinase derived from *Fusarium solani pisi* (e.g. described in WO 90/09446).

In a preferred embodiment the lipase is a variant of *Humicola lanuginosa* DSM 4109 as described in WO 00/60063. Especially preferred are the variants disclosed in the Example in WO 00/60063 with improved first wash performance., i.e., T231R+N233R; G91A+D96W+E99K+G263Q+L264A+I265T+G266D+T267A+L269N+ 270AGGFSWRRYRSAESVDKRATMTDAELEKKLNSYVQMDKEYVKNNQARS; R209P+T231R+N233R; N33Q+D96S+T231R+N233R+Q249R; E99N+N101S+T231R+ N233R+Q249R; E99N+N101S+T231R+N233R+Q249R.

Especially suitable lipases are lipases such as M1 Lipase™ and Lipomax™ (Genencor), Lipolase™ and Lipolase Ultra™, Lipex™ (Novozymes A/S), and Lipase P

"Amano" (Amano Pharmaceutical Co. Ltd.). Suitable cutinases include Lumafast™ available from Genencor Inc.

The lipases are normally incorporated in the detergent composition at a level of from 0.000001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.00001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.0001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.001% to 0.2% of enzyme protein by weight of the composition.

Amylases: Any amylase (alpha and/or beta) suitable for use in alkaline solutions can be used. Suitable amylases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. Amylases include, for example, alpha-amylases obtained from a special strain of *B. licheniformis*, described in more detail in GB 1,296,839. Commercially available amylases are Natalase™, Termamyl™ Ultra, Duramyl™, Termamyl™, Fungamyl™ and BAN™ (available from Novozymes A/S) and Rapidase™ and Maxamyl P™ (available from Genencor Inc.).

In a preferred embodiment the alpha-amylase is derived from *Bacillus* sp. strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 9375. Especially preferred are the alpha-amylases shown in SEQ ID NOS 1 and 2 of WO 95/26397.

In another preferred embodiment the alpha-amylase is the AA560 alpha-amylase derived from *Bacillus* sp. DSM 12649 disclosed as SEQ ID NO: 2 in WO 00/60060 (hereby incorporated by reference). Especially preferred are variants of the AA560 alpha-amylase, including the AA560 variant disclosed in Example 7 and 8 (hereby incorporated by reference).

The amylases are normally incorporated in the detergent composition at a level of from 0.000001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.00001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.0001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.001% to 0.2% of enzyme protein by weight of the composition.

Cellulases: Any cellulase suitable for use in alkaline solutions can be used. Suitable cellulases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. Suitable cellulases are disclosed in US 4,435,307, which discloses fungal cellulases produced from *Humicola insolens*. Especially suitable cellulases are the cellulases having colour care benefits. Examples of such cellulases are cellulases described in European patent application No. 0 495 257.

In a preferred embodiment the cellulase is a *Thielavia terrestris* cellulase, preferably the cellulase disclosed in SEQ ID NO: 9 in WO 96/29397 and SEQ ID NO: 9 herein or an

enzyme with at least 70% identity thereto. In a preferred embodiment cellulase is the *Thielavia terrestris* variant disclosed in Example 1 of WO 98/12307.

Commercially available cellulases include Celluzyme® produced by a strain of *Humicola insolens*, Carezyme® and Renozyme® (Novozymes A/S), and KAC-500(B)™ (Kao Corporation). Cellulases are normally incorporated in the detergent composition at a level of from 0.000001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.00001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.0001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.001% to 0.2% of enzyme protein by weight of the composition.

Mannanases: Any mannanase suitable for use in alkaline solutions can be used. Suitable mannanases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included.

In a preferred embodiment the mannanase is derived from a strain of the genus *Bacillus*, especially *Bacillus* sp. I633 disclosed in positions 31-330 of SEQ ID NO:2 or in SEQ ID NO: 5 of WO 99/64619 or *Bacillus agaradhaerens*, for example from the type strain DSM 8721. Further, suitable mannanases are Purabrite available from Genencor Inc. and Mannaway® produced by Novozymes A/S.

Pectate lyase: Any pectate lyase suitable for use in alkaline solutions can be used. Suitable pectate lyases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included.

In a preferred embodiment the pectate lyase is derived from a strain of the genus *Bacillus*, especially a strain of *Bacillus subtilis*, especially *Bacillus subtilis* DSM14218 disclosed in SEQ ID NO:2 or a variant thereof disclosed in Example 6 of WO 02/092741.

Peroxidases/Oxidases: Peroxidase enzymes are used in combination with hydrogen peroxide or a source thereof (e.g. a percarbonate, perborate or persulfate). Oxidase enzymes are used in combination with oxygen. Both types of enzymes are used for "solution bleaching", i.e. to prevent transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, preferably together with an enhancing agent as described in e.g. WO 94/12621 and WO 95/01426. Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically or genetically modified mutants are included.

Peroxidase and/or oxidase enzymes are normally incorporated in the detergent composition at a level of from 0.000001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.00001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.0001% to 0.5% of enzyme protein by weight

of the composition, even more preferably at a level of from 0.001% to 0.2% of enzyme protein by weight of the composition.

Mixtures of the above mentioned enzymes are encompassed herein, in particular a mixture of two, three, four, five, six or more different enzymes, for example a protease, an amylase, a lipase and a cellulase.

The enzyme of the invention, or any other enzyme incorporated in the detergent composition, is normally incorporated in the detergent composition at a level from 0.000001% to 2% of enzyme protein by weight of the composition, preferably at a level from 0.00001% to 1% of enzyme protein by weight of the composition, more preferably at a level from 0.0001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level from 0.001% to 0.2% of enzyme protein by weight of the composition.

Enzymes may be incorporated in the form of liquid solutions or granulates. Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S, now Novozymes A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonyl-phenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591.

<u>Detergent composition examples:</u> The detergent composition according to the invention can be in liquid, paste, gels, bars, tablet or granular forms.

Granular compositions according to the present invention can also be in "compact form", i.e. they may have a relatively higher density than conventional granular detergents, i.e. form 550 to 950 g/l; in such case, the granular detergent compositions according to the present invention will contain a lower amount of "Inorganic filler salt", compared to conventional granular detergents; typical filler salts are alkaline earth metal salts of sulphates and chlorides, typically sodium sulphate; "Compact" detergent typically comprise not more than 10% filler salt. The liquid compositions according to the present invention can also be in "concentrated form", in such case, the liquid detergent compositions according to the present invention will contain a lower amount of water, compared to conventional liquid detergents. Typically, the water content of the concentrated liquid detergent is less than 30%, more preferably less than 20%, most preferably less than 10% by weight of the detergent compositions.

The compositions of the invention may for example, be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the pretreatment of stained fabrics, rinse added fabric softener compositions, and compositions for use in general household hard surface cleaning operations and machine or hand dishwashing operations.

The following examples are meant to exemplify compositions for the present invention, but are not necessarily meant to limit or otherwise define the scope of the invention.

In the detergent compositions, the abbreviated component identifications have the following meanings:

LAS:	Sodium linear C_{12} alkyl benzene sulphonate
TAS:	Sodium tallow alkyl sulphate
XYAS:	Sodium C_{1X} - C_{1Y} alkyl sulfate
SS:	Secondary soap surfactant of formula 2-butyl octanoic acid
25EY:	A C_{12} - C_{15} predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide
45EY:	A C_{14} - C_{15} predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide
XYEZS:	C_{1X} - C_{1Y} sodium alkyl sulfate condensed with an average of Z moles of ethylene oxide per mole
Nonionic:	C_{13} - C_{15} mixed ethoxylated/propoxylated fatty alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5 sold under the tradename Plurafax LF404 by BASF Gmbh
CFAA:	C_{12} - C_{14} alkyl N-methyl glucamide
TFAA:	C_{16} - C_{18} alkyl N-methyl glucamide
Silicate:	Amorphous Sodium Silicate (SiO_2:Na_2O ratio = 2.0)
NaSKS-6:	Crystalline layered silicate of formula δ-$Na_2Si_2O_5$
Carbonate:	Anhydrous sodium carbonate
Phosphate:	Sodium tripolyphosphate
MA/AA:	Copolymer of 1:4 maleic/acrylic acid, average molecular weight about 80,000
Polyacrylate:	Polyacrylate homopolymer with an average molecular weight of 8,000 sold under the tradename Sokalan PA30 by BASF Gmbh

Zeolite A:	Hydrated Sodium Aluminosilicate of formula $Na_{12}(AlO_2SiO_2)_{12}.27H_2O$ having a primary particle size in the range from 1 to 10 micrometers
Citrate:	Tri-sodium citrate dihydrate
Citric:	Citric Acid
Perborate:	Anhydrous sodium perborate monohydrate bleach, empirical formula $NaBO_2.H_2O_2$
PB4:	Anhydrous sodium perborate tetrahydrate
Percarbonate:	Anhydrous sodium percarbonate bleach of empirical formula $2Na_2CO_3.3H_2O_2$
TAED:	Tetraacetyl ethylene diamine
CMC:	Sodium carboxymethyl cellulose
DETPMP:	Diethylene triamine penta (methylene phosphonic acid), marketed by Monsanto under the Tradename Dequest 2060
PVP:	Polyvinylpyrrolidone polymer
EDDS:	Ethylenediamine-N, N'-disuccinic acid, [S,S] isomer in the form of the sodium salt
Suds Suppressor:	25% paraffin wax Mpt 50°C, 17% hydrophobic silica, 58% paraffin oil
Granular Suds suppressor:	12% Silicone/silica, 18% stearyl alcohol, 70% starch in granular form
Sulphate:	Anhydrous sodium sulphate
HMWPEO:	High molecular weight polyethylene oxide
TAE 25:	Tallow alcohol ethoxylate (25)

Detergent Example I

A granular fabric cleaning composition in accordance with the invention may be prepared as follows:

Sodium linear C_{12} alkyl benzene sulfonate	6.5
Sodium sulfate	15.0
Zeolite A	26.0
Sodium nitrilotriacetate	5.0
Enzymes (incl. endoglucanase)	0.1

PVP	0.5
TAED	3.0
Boric acid	4.0
Perborate	18.0
Phenol sulphonate	0.1
Minors	Up to 100

Detergent Example II

A compact granular fabric cleaning composition (density 800 g/l) in accord with the invention may be prepared as follows:

45AS	8.0
25E3S	2.0
25E5	3.0
25E3	3.0
TFAA	2.5
Zeolite A	17.0
NaSKS-6	12.0
Citric acid	3.0
Carbonate	7.0
MA/AA	5.0
CMC	0.4
Enzyme (incl. endo-glucanase)	0.1
TAED	6.0
Percarbonate	22.0
EDDS	0.3
Granular suds suppressor	3.5
water/minors	Up to 100%

Detergent Example III

Granular fabric cleaning compositions in accordance with the invention which are especially useful in the laundering of coloured fabrics were prepared as follows:

LAS	10.7	-
TAS	2.4	-
TFAA	-	4.0
45AS	3.1	10.0
45E7	4.0	-

25E3S	-	3.0
68E11	1.8	-
25E5	-	8.0
Citrate	15.0	7.0
Carbonate	-	10
Citric acid	2.5	3.0
Zeolite A	32.1	25.0
Na-SKS-6	-	9.0
MA/AA	5.0	5.0
DETPMP	0.2	0.8
Enzyme (incl. endo-glucanase)	0.10	0.05
Silicate	2.5	-
Sulphate	5.2	3.0
PVP	0.5	-
Poly (4-vinylpyridine)-N-Oxide/copolymer of vinyl-imidazole and vinyl-pyrrolidone	-	0.2
Perborate	1.0	-
Phenol sulfonate	0.2	-
Water/Minors	Up to 100%	

Detergent Example IV

Granular fabric cleaning compositions in accordance with the invention which provide "Softening through the wash" capability may be prepared as follows:

45AS	-	10.0
LAS	7.6	-
68AS	1.3	-
45E7	4.0	-
25E3	-	5.0
Coco-alkyl-dimethyl hydroxy-ethyl ammonium chloride	1.4	1.0
Citrate	5.0	3.0
Na-SKS-6	-	11.0
Zeolite A	15.0	15.0
MA/AA	4.0	4.0
DETPMP	0.4	0.4

Perborate	15.0	-
Percarbonate	-	15.0
TAED	5.0	5.0
Smectite clay	10.0	10.0
HMWPEO	-	0.1
Enzyme (incl. endo-glucanase)	0.10	0.05
Silicate	3.0	5.0
Carbonate	10.0	10.0
Granular suds suppressor	1.0	4.0
CMC	0.2	0.1
Water/Minors	Up to 100%	

Detergent Example V

Heavy duty liquid fabric cleaning compositions in accordance with the invention may be prepared as follows:

	I	II
LAS acid form	-	25.0
Citric acid	5.0	2.0
25AS acid form	8.0	-
25AE2S acid form	3.0	-
25AE7	8.0	-
CFAA	5	-
DETPMP	1.0	1.0
Fatty acid	8	-
Oleic acid	-	1.0
Ethanol	4.0	6.0
Propanediol	2.0	6.0
Enzyme (incl.endo-glucanase)	0.10	0.05
Coco-alkyl dimethyl hydroxy ethyl ammonium chloride	-	3.0
Smectite clay	-	5.0
PVP	2.0	-
Water / Minors	Up to 100%	

Use of detergents: The enzyme composition of the invention may be useful in a detergent composition for household or industrial laundering of textiles and garments, and in a process for machine wash treatment of fabrics comprising treating the fabrics during one or more washing cycle of a machine washing process with a washing solution containing the enzyme or enzyme preparation of the invention.

The enzyme composition of the invention may also be useful in a detergent composition for household or industrial dish or cutlery or other hard surface washing, and in a process for treatment of dishes, cutlery etc. comprising a treatment with a washing solution containing the enzyme or enzyme preparation of the invention.

Typically, the detergent composition used in the washing process comprises conventional ingredients such as surfactants (anionic, nonionic, zwitterionic, amphoteric), builders, bleaches (perborates, percarbonates or hydrogen peroxide) and other ingredients, e.g. as described in WO 97/01629 which is hereby incorporated by reference in its entirety.

The endo-glucanase of the invention provides advantages such as improved stain removal and decreased soil redeposition. Certain stains, for example certain food stains, contain beta-glucans which make complete removal of the stain difficult to achieve. Also, the cellulosic fibres of the fabrics may possess, particularly in the "non-crystalline" and surface regions, glucan polymers that are degraded by this enzyme. Hydrolysis of such glucans, either in the stain or on the fabric, during the washing process decreases the binding of soils onto the fabrics.

Household laundry processes are carried out under a range of conditions. Commonly, the washing time is from 5 to 60 minutes and the washing temperature is in the range 10 - 60°C, most commonly from 20 - 40°C. Prolonged soaking is commonly used. The washing solution is normally neutral or alkaline, most commonly with pH 5 - 11.5. Bleaches are commonly used, particularly for laundry of white fabrics and in the washing of hard surfaces. These bleaches are commonly the peroxide bleaches, such as sodium perborate, sodium percarbonate or hydrogen peroxide.

MATERIALS & METHODS

Strains and donor organism

The *Bacillus sp.* DSM 12648 mentioned above comprises the endo-glucanase encoding DNA sequence shown in SEQ ID NO:1.

B.subtilis PL2306: This strain is the *B.subtilis* DN1885 with disrupted apr and npr genes (Diderichsen, B., Wedsted, U., Hedegaard, L., Jensen, B. R., Sjøholm, C. (1990) Cloning of aldB, which encodes alpha-acetolactate decarboxylase, an exoenzyme from *Bacillus brevis*. J. Bacteriol., 172, 4315-4321) disrupted in the transcriptional unit of the known *Bacillus*

subtilis cellulase gene, resulting in cellulase negative cells. The disruption was performed essentially as described in Eds. A.L. Sonenshein, J.A. Hoch and Richard Losick (1993) *Bacillus subtilis* and other Gram-Positive Bacteria, American Society for microbiology, p.618.

Competent cells were prepared and transformed as described by Yasbin, R.E., Wilson, G.A. and Young, F.E. (1975) Transformation and transfection in lysogenic strains of *Bacillus subtilis*: evidence for selective induction of prophage in competent cells. J. Bacteriol, 121:296-304.

General molecular biology methods

Unless otherwise stated all the DNA manipulations and transformations were performed using standard methods of molecular biology (Sambrook et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor lab., Cold Spring Harbor, NY; Ausubel, F. M. et al. (eds.) "Current protocols in Molecular Biology". John Wiley and Sons, 1995; Harwood, C. R., and Cutting, S. M. (eds.) "Molecular Biological Methods for Bacillus". John Wiley and Sons, 1990).

Enzymes for DNA manipulations were used according to the manufacturer's instructions (e.g. restriction endonucleases, ligases etc. are obtainable from New England Biolabs, Inc.).

Plasmids

pMOL944. This plasmid is a pUB110 derivative essentially containing elements making the plasmid propagate in *Bacillus subtilis*, kanamycin resistance gene and having a strong promoter and signal peptide cloned from the amyL gene of *B.licheniformis* ATCC14580. The signal peptide contains a SacII site making it convenient to clone the DNA encoding the mature part of a protein in-fusion with the signal peptide. This results in the expression of a Preprotein which is directed towards the exterior of the cell.

The plasmid was constructed by means of ordinary genetic engineering and is briefly described in the following.

<u>Construction of pMOL944:</u>

The pUB110 plasmid (McKenzie, T. et al., 1986, Plasmid 15:93-103) was digested with the unique restriction enzyme NciI. A PCR fragment amplified from the amyL promoter encoded on the plasmid pDN1981 (P.L. Jørgensen et al., 1990, Gene, 96, p37-41.) was digested with NciI and inserted in the NciI digested pUB110 to give the plasmid pSJ2624.

The two PCR primers used have the following sequences:

LWN5494 (SEQ ID NO:5)

5´-GTCGCCGGGGCGGCCGCTATCAATTGGTAACTGTATCTCAGC -3´

LWN5495 (SEQ ID NO:6)

5´-GTCGCCCGGGAGCTCTGATCAGGTACCAAGCTTGTCGACCTGCAGAA TGAGGCAGCAAGAAGAT -3´

The primer #LWN5494 inserts a NotI site in the plasmid.

The plasmid pSJ2624 was then digested with SacI and NotI and a new PCR fragment amplified on amyL promoter encoded on the pDN1981 was digested with SacI and NotI and this DNA fragment was inserted in the SacI-NotI digested pSJ2624 to give the plasmid pSJ2670.

This cloning replaces the first amyL promoter cloning with the same promoter but in the opposite direction. The two primers used for PCR amplification have the following sequences:

#LWN5938 (SEQ ID NO:7)

5`-GTCGGCGGCCGCTGATCACGTACCAAGCTTGTCGACCTGCAGAATG AGGCAGCAAGAAGAT -3´

#LWN5939 (SEQ ID NO:8)

5`-GTCGGAGCTCTATCAATTGGTAACTGTATCTCAGC -3´

The plasmid pSJ2670 was digested with the restriction enzymes PstI and BclI and a PCR fragment amplified from a cloned DNA sequence encoding the alkaline amylase SP722 (Patent # WO9526397-A1) was digested with PstI and BclI and inserted to give the plasmid pMOL944. The two primers used for PCR amplification have the following sequence:

#LWN7864 (SEQ ID NO:9)

5` -AACAGCTGATCACGACTGATCTTTTAGCTTGGCAC-3´

#LWN7901 (SEQ ID NO:10)

5` -AACTGCAGCCGCGGCACATCATAATGGGACAAATGGG -3´

The primer #LWN7901 inserts a SacII site in the plasmid.

Genomic DNA Preparation

The strain DSM 12648 was propagated in liquid medium 2xTY containing 1% carboxymethyl-cellulose + (0.1M Na2CO3 + 0.1M NaHCO3 separately autoclaved and added aseptically after cooling to room temperature). After 16 hours of incubation at 30°C and 300 rpm, the cells were harvested, and genomic DNA was isolated by the method described by *Pitcher et al.* [*Pitcher, D. G., Saunders, N. A., Owen, R. J*; Rapid extraction of bacterial genomic DNA with guanidium thiocyanate; Lett Appl Microbiol 1989, 8:151-156].

Media

TY (as described in Ausubel, F. M. et al. (eds.): "Current protocols in Molecular Biology", John Wiley and Sons, 1995).

2xTY (as described in Ausubel, F. M. et al. (eds.): "Current protocols in Molecular Biology", John Wiley and Sons, 1995).

LB agar (as described in Ausubel, F. M. et al. (eds.): "Current protocols in Molecular Biology", John Wiley and Sons, 1995).

LBPG is LB agar supplemented with 0.5% Glucose and 0.05 M potassium phosphate, pH 7.0

AZCL-HE-cellulose is added to LBPG-agar to 0.5 % AZCL- HE-cellulose is from Megazyme, Australia.

BPX media is described in EP 0 506 780 (WO 91/09129).

Cal 18-2 media is described in patent application WO 00/75344 A1).

<u>Test for endo-glucanase activity</u>

The purpose of this test, used in combination with the "test for anti-redeposition effect" described below, is to determine if an enzyme is an endo-glucanase with anti-redeposition effect, according to this invention.

The test is made by determining the color released from an insoluble, colored, glucan substrate over a reaction time of 30 minutes at a temperature of 40°C.

Enzyme sample: The enzyme sample for testing is a solution of the enzyme protein with a concentration of 0.1mg /ml. Standard biochemical techniques can be used to verify the purity and determine the protein concentration of the sample.

Buffer: Prepare a 0.05M phosphate buffer solution with pH 7.0 from $NaH_2PO_4.2H_2O$ and NaOH and add 1g/l nonionic surfactant (e.g. Berol 537, from Akzo Nobel).

Test method: Transfer 6 ml buffer into two test tubes. To one tube add 50µl of the enzyme sample. No enzyme is added to the second tube. Add one beta-glucazyme tablet (supplied by Megazyme, Ireland, catalogue number T-BGZ) to each tube. Mix the contents of the tubes for 10 seconds with a vortex mixer, then place the tubes in a 40°C water bath. After 10 minutes and after 20 minutes, mix the contents of the tubes by inverting the tubes. After 30 minutes, mix the contents of the tubes by inverting the tubes and then filter the contents of the tubes through a Whatman GF/C 9cm filter, collecting the filtrates in clean tubes. Measure the color released as OD at 590 nm using a spectrophotometer. Calculate deltaOD by subtracting the result with no enzyme from the result with enzyme.

If deltaOD > 0.2 then the enzyme is an endo-glucanase.

Test for anti-redeposition effect

The purpose of this test, used in combination with the "test for endo-glucanase activity" described above, is to determine if an enzyme is an endo-glucanase with anti-redeposition effect, according to this invention.

The anti-redeposition effect is measured by a wash test. The anti-redeposition wash test is made by washing samples of soiled cotton fabric and samples of clean cotton fabric, both together, in a small-scale wash test apparatus. After the washing the soil on the originally clean cotton fabric is evaluated by light reflectance.

Enzyme sample: The enzyme sample for testing is a solution of the enzyme protein with a concentration of 0.1mg /ml. Standard biochemical techniques can be used to verify the purity and determine the protein concentration of the sample.

Cotton fabric: #2003 white woven 100% cotton fabric, supplied by Tanigashira, 4-11-15 Komatsu Yodogawa-ku, Osaka, 533-0004, Japan. The new cotton fabric is pre-washed three times before use in the wash test. The pre-washing is done using a European household front-loader washing machine, and using a standard 40°C wash process. LAS (Surfac® SDBS80 sodium alkylbenzene sulfonate, 80%) is added to the wash water at concentration 0.5 g per liter and the wash solution pH is adjusted to 10 by addition of sodium carbonate. After the pre-washing the fabric is dried in a tumbler drier. Swatches of the pre-washed cotton fabric, size 5x5cm, weight approximately 0.3g each, are cut out and these swatches are used for the wash tests.

Soiled cotton swatches: Swatches of the pre-washed #2003 fabric, prepared as above, are soiled as follows. A suspension of 210 mg carbon black ("carbon for detergency tests", supplied by Sentakukagaku-kyokai, 2-11-1 Shimomaruko Ohta-ku, Tokyo 146-8620, Japan) is prepared in 75 ml tetrachloroethylene (Fluka, cat. nr. 86972) by strong stirring. The cotton swatches are placed flat on a horizontal metal surface. 300µl of the carbon suspension is pipetted onto the centre of each cotton swatch. The soiled cotton swatches are allowed to dry at room temperature overnight.

Detergent solutions: Detergent solutions are prepared as follows. To prepare 1 liter of solution, dissolve in deionised water 0.5g sodium carbonate and 1.0g sodium hydrogen carbonate and add 2 ml of a solution containing 117.8 g/l $CaCl_2.2H_2O$ and 54.3 g/l $MgCl_2.6H_20$. This calcium/magnesium addition provides a water hardness of 12 °dH. Add 0.5g LAS (Surfac SDBS80 sodium alkylbenzene sulfonate, 80%, supplied by Surfachem, UK or an equivalent material) and adjust the final volume to 1 liter. Adjust the pH to 9.5 (e.g. by addition of sodium carbonate)

Wash tests: Three soiled swatches (prepared as described) and three clean swatches (of the same pre-washed #2003 cotton) are washed in a Mini-Terg-O-Tometer machine. The Mini-Terg-O-Tometer is a small-scale version of the Terg-O-Tometer test

washing machine described in Jay C. Harris, "Detergency Evaluation and Testing", Interscience Publishers Ltd. (1954) pp. 60-61. The following conditions are used:

Beaker size	250ml
Wash solution volume	100ml
Wash temperature	40°C
Wash time	30 minutes
Agitation	150 rpm

The detergent solutions are pre-warmed to 40°C before starting the test. The cotton swatches and 100µl of the enzyme sample are added at the start of the 30 minute wash period. The reference test, with no added enzyme, is started at the same time.

After the wash, the fabric swatches are rinsed for 5 minutes under running tap water, then spread out flat and allowed to air dry at room temperature overnight.

Instrumental evaluations: Light reflectance evaluation of the originally clean fabric swatches is done using a Macbeth Color Eye 7000 reflectance spectrophotometer. The measurements are made at 500nm. The UV filter is not included. Measurements are made on the front and back of each swatch. An average result for reflectance (R, 500nm) for the three originally clean swatches is then calculated from the six measurements.

The anti-redeposition effect of a given enzyme sample is calculated as:

(R, 500nm with the enzyme) – (R, 500nm no enzyme added)

The enzyme is an endo-glucanase with anti-redeposition effect according to the invention if the anti-redeposition effect obtained is >7.5, <u>and</u> the result in the test for endo-glucanase activity, above, is >0.2.

<u>**LAS stability test**</u>

The test is made by comparing the color released from an insoluble colored cellulase substrate over a reaction time of 60 minutes at a temperature of 40°C when the solution contains a buffer but no surfactant and when the solution contains a buffer and LAS. If the enzyme is stable during the 60 minute reaction time then the color released is greater than if the enzyme is not stable, because the stable enzyme will continue to degrade the substrate whereas the non-stable enzyme will not continue to degrade the substrate. In the context of the present invention, if the result with LAS is > 50% of the result without LAS then the enzyme is LAS stable.

The details of the test method are as follows.

Buffer (no LAS):

Prepare a 0.05M phosphate buffer solution with pH 7.0 from $NaH_2PO_4.2H_2O$ and NaOH.

Buffer (with LAS):

Dissolve 1.0g LAS in 1 liter of the above buffer. The LAS is Surfac® SDBS80 sodium alkylbenzene sulfonate, 80% (supplied by Surfachem, UK) or an equivalent material..

Test method:

Prepare a dilution of the enzyme to be tested in deionised water. The concentration of the enzyme is such that the result for deltaOD (no LAS), calculated as described below, is in the range 0.2 to 0.5.

Transfer 6 ml of the buffer (no LAS) into two test tubes. Add 150µl of the enzyme dilution into one tube. No enzyme is added to the other tube. Add one Cellazyme® C tablet (supplied by Megazyme, Ireland, catalogue number T-CCZ) into both tubes. Mix strongly for about 10 seconds using a vortex mixer. Place both tubes in a 40°C water bath. After 15, 30 and 45 minutes, mix the contents of the tubes by inverting the tubes. After 60 minutes, mix the contents of the tubes by inverting the tubes and then filter the contents through a 9cm diameter Whatman® GF/C filter, collecting the filtrate in a clean tube. Measure the OD of the filtrate at 590 nm using a spectrophotometer. Calculate deltaOD (no LAS) by subtracting the result with no enzyme from the result with enzyme.

Then repeat the test using the same concentration of the enzyme and with 6 ml buffer (with LAS) instead of 6 ml buffer (no LAS). Calculate deltaOD (with LAS) by subtracting the result with no enzyme from the result with enzyme.

Calculate the LAS stability %:

(deltaOD (with LAS) / deltaOD (no LAS)) * 100

Wash tests with the endo-glucanase in combination with other enzymes

This procedure is used to determine the enzyme detergency benefit values, see Examples 6 – 11 below.

The wash tests are made by washing samples of soiled cotton fabric and samples of clean cotton fabric, both together, in a small-scale wash test apparatus. After the washing the soil on the cotton fabric is evaluated by light reflectance. Both the originally soiled cotton fabric and the originally clean cotton fabric samples are evaluated.

Cotton fabric: #2003 white woven 100% cotton fabric, supplied by Tanigashira, 4-11-15 Komatsu Yodogawa-ku, Osaka, 533-0004, Japan. The new cotton fabric is pre-washed three times before use in the wash test. The pre-washing is done using a European household

front-loader washing machine, and using a standard 40°C wash process. LAS (Surfac® SDBS80 sodium alkylbenzene sulfonate, 80%) is added to the wash water at concentration 0.5 g per liter and the wash solution pH is adjusted to 10 by addition of sodium carbonate. After the pre-washing the fabric is dried in a tumbler drier. Swatches of the pre-washed cotton fabric, size 5x5cm, weight approximately 0.3g each, are cut out and these swatches are used for the wash tests.

Soiled cotton swatches: These are prepared from the 5x5cm swatches described above.

Wash tests: Three soiled swatches and three clean swatches are washed in a Mini-Terg-O-Tometer machine. The Mini-Terg-O-Tometer is a small-scale version of the Terg-O-Tometer test washing machine described in Jay C. Harris, "Detergency Evaluation and Testing", Interscience Publishers Ltd. (1954) pp. 60-61. The following conditions are used:

Beaker size	250ml
Wash solution volume	100ml
Wash temperature	40°C
Wash time	30 minutes
Agitation	150 rpm

The detergent solutions are pre-warmed to 40°C before starting the test. The fabric and the enzymes are added at the start of the 30 minute wash period. After the wash, the fabric swatches are rinsed for 5 minutes under running tap water, then spread out flat and allowed to air dry at room temperature overnight.

Instrumental evaluations: Light reflectance evaluation of the fabric swatches is done using a Macbeth Color Eye 7000 reflectance spectrophotometer. The measurements are made at 500nm. The UV filter is not included. Measurements are made on the front and back of each swatch. The soiled swatches are measured in the centre of the soiled area. Average results for reflectance (R, 500nm) for the soiled swatches and for the clean swatches are then calculated from the six measurements on each type.

Detergent solutions: Detergent solutions are prepared as follows: To prepare 1 liter of solution, dissolve in deionised water 0.5g sodium carbonate and 1.0g sodium hydrogen carbonate and add 2 ml of a solution containing 117.8 g/l $CaCl_2.2H_2O$ and 54.3 g/l $MgCl_2.6H_20$. This calcium/magnesium addition provides a water hardness of 12 °dH. Add nonionic surfactant (Berol® 537, Akzo Nobel) and/or LAS (Surfac® SDBS80 sodium alkylbenzene sulfonate, 80%) and adjust the final volume to 1 liter. Adjust the pH to either pH 9.5 (by addition of sodium carbonate) or to pH 7.5 (by addition of 10% citric acid solution).

The concentration of LAS and of nonionic surfactant and the detergent solution pH are specified below for each wash test.

Enzyme addition: The enzymes to be tested are pre-dissolved at known concentrations in water, and the required amount of enzyme is added to the detergent solution at the start of the wash process.

Calculation of enzyme detergency benefit: The enzyme detergency benefit is a measure of how much more clean the swatches, both the originally soiled and the originally clean, become as a result of including enzymes in the wash test. The enzyme detergency benefit is calculated as follows:

After the wash test the average R, 500nm value for the soiled swatches is R, soiled.
After the wash test the average R, 500nm value for the clean swatches is R, clean.
The enzyme detergency benefit from a wash test with enzymes is the sum of R, soiled + R, clean with enzymes minus the sum of R, soiled + R, clean with no added enzyme.

The enzyme detergency benefit value determined in this way is a combined measure both of the removal of soil from the fabric and of the redeposition of soil onto the fabric. Thus the enzyme detergency benefit value can have values that are negative or positive. The enzyme detergency benefit value can be used to compare the performance of different enzymes. The highest positive detergency benefit value is the preferred result.

EXAMPLE 1

Cloning and expression of endo-glucanase gene from *Bacillus sp.*

<u>Sub-cloning and expression of mature endo-glucanase in *B.subtilis*.</u>

The endo-glucanase encoding DNA sequence of the invention was PCR amplified using the PCR primer set consisting of these two oligo-nucleotides:

168684 (SEQ ID NO:11)
5'-CAT TCT GCA G<u>CC GCG G</u>CA GCA GAA GGA AAC ACT CGT GAA GAC-3'

168685 (SEQ ID NO:12)
5'-GCG TTG AGA CGC <u>GCG GCC GC</u>T TAC TCT TCT TTC TCT TCT TTC TC-3'

Restriction sites SacII and NotI are underlined.

The oligonucleotides were used in a PCR reaction in HiFidelity™ PCR buffer (Boehringer Mannheim, Germany) supplemented with 200 micro M of each dNTP, 2.6 units of HiFidelity™ Expand enzyme mix and 200 pmol of each primer. Chromosomal DNA isolated from *Bacillus sp.* DSM12648 as described above was used as template.

The PCR reaction was performed using a DNA thermal cycler (Landgraf®, Germany). One incubation at 94°C for 1 min followed by ten cycles of PCR performed using a cycle profile of denaturation at 94°C for 15 sec, annealing at 60°C for 60 sec, and extension at 72°C for 120sec, followed by twenty cycles of denaturation at 94°C for 15 sec, 60°C for 60 sec and 72°C for 120 sec (at this elongation step 20 sec are added every cycle). 5 µl aliquots of the amplification product was analysed by electrophoresis in 0.7 % agarose gels (NuSieve®, FMC). The appearance of a DNA fragment size 2.4 kb indicated proper amplification of the gene segment.

Subcloning of PCR fragment:

45 microL aliquots of the PCR products generated as described above were purified using QIAquick® PCR purification kit (Qiagen®, USA) according to the manufacturer's instructions. The purified DNA was eluted in 50 microL of 10mM Tris-HCl, pH 8.5.

5 µg of pMOL944 and 25 microL of the purified PCR fragment was digested with SacII and NotI, electrophoresed in 0.7 % agarose gels (NuSieve®, FMC), the relevant fragments were excised from the gels, and purified using QIAquick® Gel extraction Kit (Qiagen®, USA) according to the manufacturer's instructions. The isolated PCR DNA fragment was then ligated to the SacII-NotI digested and purified pMOL944. The ligation was performed overnight at 16°C using 0.5 micro g of each DNA fragment, 1 U of T4 DNA ligase and T4 ligase buffer (Boehringer Mannheim, Germany).

The ligation mixture was used to transform competent *B. subtilis* PL2306. The transformed cells were plated onto LBPG-10 micro g/ml of kanamycin-agar plates. After 18 hours incubation at 37°C colonies were seen on the plates. Several clones were analyzed by isolating plasmid DNA from overnight culture broths.

One such positive clone was re-streaked several times on agar plates as used above; this clone was called MB1181-7. The clone MB1181-7 was grown overnight in TY-10micro g/mL kanamycin at 37°C, and next day 1 ml of cells were used to isolate a plasmid from the cells using the Qiaprep® Spin Plasmid Miniprep Kit #27106 according to the manufacturers recommendations for *B. subtilis* plasmid preparations. This DNA was sequenced and revealed a DNA sequence identical to the endo-glucanase gene in SEQ ID NO:1 bp 1-2322 encoding the mature endo-glucanase. The derived protein sequence is represented in SEQ ID NO: 2.

EXAMPLE 2

Expression and recovery of the endo-glucanase from *Bacillus sp.* DSM 12648

MB1181-7 obtained as described in Example 1 was grown in 15 x 200 ml Cal-18-2 media with 10 microg/mL of kanamycin, in 500 mL two-baffled shake flasks, for 4 days at 37°C

at 300 rpm, whereby about 2500 ml of culture broth was obtained. The culture fluid was flocculated by adding 50% $CaCl_2$ (10 ml per liter of culture broth) together with 11% sodium aluminate (10 mL per liter of culture broth), maintaining the pH between 7.0 and 7.5 by adding 20% formic acid. Cationic agent Superfloc® C521 (25 mL of a 10% v/v dilution per liter of culture broth) and anionic agent Superfloc® A130 (75 ml of a 0.1% w/v dilution in water per liter of culture broth) was added during agitation to complete the flocculation. The flocculated material was separated by centrifugation using a Sorval® RC 3B centrifuge at 10000 rpm for 30 min at 6°C. The resulting supernatant contained the endo-glucanase activity.

The supernatant was clarified using Whatman glass filters GF/D and C. Then ultra-filtration was used to concentrate and reduce the ionic strength of the solution. The ultra-filtration membrane was Filtron® UF with a cut-off of 10 kDa. After ultra-filtration the solution had conductivity < 3mS/cm. The pH was adjusted to pH 8.0.

Anion-exchange chromatography on Q-Sepharose® was then used for additional purification. The solution from ultra-filtration was applied to a 300 mL column containing Q-Sepharose® (Pharmacia) equilibrated with a buffer of 25 mmol Tris pH 8.0. The endo-glucanase bound to the Q-Sepharose®, and was then eluted using a 0.5 M NaCl gradient. The fractions with high endo-glucanase activity were pooled. The endo-glucanase activity of the final pooled endo-glucanase solution was approximately 1000 ECU per mL.

The activity units, ECU, are determined by method SM-0302, which is available from Novozymes A/S on request. In the ECU method the ability of the enzyme sample to reduce the viscosity of a solution of carboxymethyl-cellulose is determined, and the result is given in ECU. Conditions: CMC type 7LFD from Hercules, pH7.5 in 0.1M phosphate buffer, CMC concentration 31.1 g per liter, reaction at 40°C for 30 minutes. A vibration viscosimeter such as MIVI 3000, Sofraser®, France is used to measure the viscosity.

EXAMPLE 3: Stain removal and anti-redeposition effect

This test demonstrates the stain removal and anti-redeposition effects of the endo-glucanase obtained in Example 2. Additionally this test demonstrates that the enzyme performance is essentially unchanged when sodium perborate bleach is included.

Cotton swatches are stained with beta-glucan (from barley) plus carbon black. Soiled swatches are washed together with clean swatches. After washing the swatches are rinsed and dried. The soil removal from the soiled switches and the soil redeposition onto the clean swatches is determined by reflectance measurements. The soil removal and soil redeposition after washing without or with addition of the endo-glucanase are compared.

Swatches: Cut from 100% cotton fabric, type #2003 (Tanigashira, Osaka, Japan), pre-washed at 40°C as a precaution to remove any water soluble contaminations, size 5x5cm, weight approximately 0.3g.

Washing equipment: Stirred beakers, beaker volume 250 ml, with temperature control by water bath heating. The equipment is the Mini-Terg-O-Tometer, a multi-beaker miniature agitator washer, as described in the Materials and Methods section.

Detergent solution: Prepared by adding the following into deionised water.

Sodium carbonate, 0.5 g per liter

Sodium bicarbonate, 0.7 g per liter

Ca^{2+}/Mg^{2+}, to give water hardness 12°dH

Anionic surfactant, Surfac® SDBS80 (sodium alkylbenzene sulphonate), 0.5 g per liter

Nonionic surfactant, Berol® 537 (Akzo Nobel), 1.0 g per liter

Sodium perborate, type SPB from wfk Testgewebe, either 0 or 1.0 g per liter

Solution pH is approximately 9.5.

Washing procedure: 100mL detergent solution is added to each beaker. The water bath temperature is 40°C. The mechanical agitators are operated at approximately 125 rpm. The detergent solutions are pre-warmed for 10 minutes and then the endo-glucanase and the swatches are added. In each case three soiled swatches (prepared as described below) and three clean swatches are added to each beaker. After washing for 30 minutes, the swatches are removed from the detergent solution, rinsed under running tap water for 5 minutes, spread flat on absorbent paper and allowed to dry.

Reflectance measurements: Made using a Macbeth® 7000 Color Eye reflectance spectrophotometer. In the case of the soiled swatches, each swatch is measured once in the center of the soiled area, then the average value is calculated. In the case of the clean swatches, each swatch is measured once on each side, then the average value is calculated. The reflectance measurements are all made at 500nm.

Soiled swatches: Soiled swatches are made using beta-glucan (from barley) and carbon black ("carbon for detergency tests" supplied by Sentaku Kagaku Kyokai, Tokyo, Japan). Dissolve about 0.67g of beta-glucan in 100 mL tap water by stirring and warming to >50°C. Add 0.33g carbon black. Blend with an UltraTurrax® T25 blender, speed 4000 rpm for 2 minutes. Apply 250 microL of the beta-glucan/carbon onto the center of each swatch. Allow to dry overnight at room temperature.

The swatches used in this example had an average reflectance value of 93.5 before soil application and 17.5 after soiling.

Endo-glucanase addition: The endo-glucanase from Example 2 was added to give an activity concentration of 0, 20 or 100 ECU per liter of detergent solution. The activity units, ECU, are determined by method SM-0302, as above.

Results: Detergent without bleach (average of reflectance measurements after washing)

Endo-glucanase added	Soiled swatches	Clean swatches
0	25.1	33.5
20 ECU per liter	35.7	46.7
100 ECU per liter	40.2	59.1

Results: Detergent with bleach (average of reflectance measurements after washing)

Endo-glucanase added	Soiled swatches	Clean swatches
0	24.6	27.7
20 ECU per liter	36.8	52.6
100 ECU per liter	39.3	63.2

The endo-glucanase increased the removal of soil from the fabric, as seen by the increased reflectance value of the stained swatches after washing with endo-glucanase as compared to the result after washing without endo-glucanase. The endo-glucanase also decreases the soil redeposition, as seen by the increased reflectance value of the clean swatches after washing with endo-glucanase. The improvements of soil removal and anti-redeposition provided by the endo-glucanase are essentially unchanged by the addition of the bleach.

EXAMPLE 4: Anti-redeposition effect

Clean cotton fabric is washed together with soiled cotton fabric in a solution of a household detergent. The wash is carried out in a Terg-O-Tometer. During the wash, soil is released from the soiled fabric into the detergent liquor. This soil can then redeposit onto the clean cotton. After washing, the cotton fabrics are rinsed and dried, and then measured with a reflectance spectrophotometer in order to detect the degree of soil redeposition.

Detergent: Powder household detergent, Asian.

Detergent concentration: 0.67g/l in water with hardness 4°dH.

1000 mL of detergent solution per T-O-T beaker.

Cotton fabric: Total of 33g fabric per T-O-T beaker, comprising suitably sized pieces of:

white woven cotton, #2003 (Tanigashira, Osaka, Japan), total weight 11g

white cotton interlock, total weight 13g

soiled cotton fabric, type EMPA101 (EMPA, Switzerland), total weight 9g.

Wash: Temperature 25°C, wash time 40 minutes, at 125 rpm. After washing the #2003 cotton is rinsed under running tap water for 10 minutes, then dried.

Reflectance measurements. The pieces of #2003 woven cotton are measured, on both sides, using a Macbeth® 7000 reflectance spectrophotometer, 500nm. The average result for measurements from each T-O-M beaker is calculated.

Enzyme addition: In this trial, the glucanase prepared as described in Example 2 was added to the detergent liquor before the start of the wash step.

Results:

Endo-glucanase added (ECU per liter)	Reflectance of #2003, at 500nm
0	76.67
0	76.05
1	81.86
5	84.30
20	84.85
50	85.99

The activity units, ECU, are determined by method SM-0302, as above. From the results it can be concluded that addition of the endo-glucanase reduces the soil redeposition.

EXAMPLE 5: LAS stability of enzyme samples

The LAS stability % was determined by the procedure described above. The following enzymes were tested:

Carezyme is the trade name for enzyme products from Novozymes A/S that contain (as the only enzyme component) the 43 kD cellulase derived from *Humicola insolens* DSM 1800 disclosed in WO 91/17243 (SEQ ID NO: 2 and hereby incorporated by reference). Detergent compositions containing this enzyme are disclosed in EP 822 973.

Renozyme is the trade name for enzyme products from Novozymes A/S that contain (as the only enzyme component) the *Thielavia terrestris* cellulase variant disclosed in Example 1 of WO 98/12307.

Endo-glucanase. This is an endo-glucanase with anti-redeposition effect as defined above. A method for preparation of this enzyme is described in Example 2.

Results:

Enzyme	LAS stability %
Carezyme	13
Renozyme	62

Endo-glucanase	83

These results show that, according to the definition used, Carezyme is not LAS stable and that both Renozyme and the endo-glucanase are LAS stable.

EXAMPLE 6: Wash test: Renozyme® and endo-glucanase

The purpose of this test was to measure the enzyme detergency benefit of Renozyme® alone and of Renozyme® in combination with endo-glucanase.

For this test the soiled swatches were prepared as follows: Swatches of the pre-washed #2003 fabric, prepared as above, were soiled as follows. A suspension of 210 mg carbon black ("carbon for detergency tests", supplied by Sentakukagaku-kyokai, 2-11-1 Shimomaruko Ohta-ku, Tokyo 146-8620, Japan) was prepared in 75 ml tetrachloroethylene (Fluka®, cat. nr. 86972) by strong stirring. The cotton swatches were placed flat on a horizontal metal surface. 300µl of the carbon suspension was pipetted onto the centre of each cotton swatch. The soiled cotton swatches were allowed to dry at room temperature overnight.

For this test the detergent composition was as described above and with the following surfactants and pH:

LAS: 0.5g per liter
Nonionic: none
pH: 9.5

Enzyme detergency benefit results:

	Renozyme® at 50 µg/l	Renozyme® at 125 µg/l
No endo-glucanase	6.1	8.1
endo-glucanase at 75µg/l	15.4	
endo-glucanase at 190 µg/l		16.9

The enzyme concentrations are specified here in terms of the equivalent concentration of the pure, catalytically active enzyme proteins, in order to avoid ambiguity resulting from activity assay procedures. Standard biochemical techniques can be used to purify and characterise the enzymes.

A suitable cellulase can be obtained from samples of the commercial product Renozyme® from Novozymes A/S or derived as described in Example 1 of WO 98/12307.

The endo-glucanase is an endo-glucanase with anti-redeposition effect as defined above. A method for preparation of this enzyme is described in Example 2.

The results show that the combinations of Renozyme® and the endo-glucanase give a higher enzyme detergency benefit than Renozyme® alone in a detergent solution that contains LAS but no other surfactants.

EXAMPLE 7: Wash test: Stainzyme® and endo-glucanase

The purpose of this test was to measure the enzyme detergency benefit of Stainzyme® alone and of Stainzyme® in combination with endo-glucanase on a soil that contains starch.

Stainzyme® is the trade name of a commercially available alpha-amylase product produced by Novozymes A/S. Stainzyme® is intended for use in detergents for laundry and hard surface cleaning.

For this test the soiled swatches were prepared as follows: Swatches of the pre-washed #2003 fabric, prepared as above, were soiled as follows. A suspension of 4.5g of potato starch ("kartoffelmel", produced by KMC kartoffelmel-centralen, DK-7400 Herning, Denmark, starch content approximately 80%) was prepared in 500ml tap water with stirring, then heated to boiling and then cooled. To 100ml of this solution, 332 mg of carbon black ("carbon for detergency tests", supplied by Sentakukagaku-kyokai, 2-11-1 Shimomaruko Ohta-ku, Tokyo 146-8620, Japan) were added, and the suspension was homogenised with an UltraTurrax® blender. The cotton swatches were placed flat on a horizontal metal surface. 250µl of the carbon/starch suspension was pipetted onto the centre of each cotton swatch. The soiled cotton swatches were allowed to dry at room temperature overnight.

For this test the detergent composition was as described above and with the following surfactants and pH:

LAS: 0.4g per liter
Nonionic: 0.2g per liter
pH: 9.5

Enzyme detergency benefit results:

	Stainzyme® at 2.1 mg/l	Stainzyme® at 5.3 mg/l
No endo-glucanase	-2.9	1.7
endo-glucanase at 75 µg/l	10.1	
endo-glucanase at 190 µg/l		13.1

The enzyme concentrations are specified here in terms of the equivalent concentration of the pure, catalytically active enzyme proteins, in order to avoid ambiguity resulting from activity assay procedures. Standard biochemical techniques can be used to purify and characterise the enzymes.

A suitable amylase can be obtained from samples of the commercial product Stainzyme® from Novozymes A/S or derived from *B. licheniformis* variants as disclosed in WO 01/66712 and WO 01/64852.

The endo-glucanase is an endo-glucanase with anti-redeposition effect as defined above. A method for preparation of this enzyme is described in Example 2.

In this test the soil contains starch. The results show that the combinations of endo-glucanase and Stainzyme® give a higher enzyme detergency benefit than Stainzyme® alone.

EXAMPLE 8: Wash test: Savinase® and endo-glucanase

The purpose of this test was to measure the enzyme detergency benefit of Savinase alone and of Savinase in combination with endo-glucanase on a soil that contains protein.

Savinase® is the trade name of subtilisin 309. It is a commercially available protease product produced by Novozymes A/S. Savinase® is intended for use in detergents for laundry and hard surface cleaning.

For this test the soiled swatches were prepared as follows: Swatches of the pre-washed #2003 fabric, prepared as above, were soiled as follows. A solution of 7.2g of gelatine (gelatine from porcine skin, Fluka®, cat. number 04055) was prepared in 100ml tap water by warming and with stirring. 10 ml of this solution was diluted to 50ml with water and 165 mg of carbon black ("carbon for detergency tests", supplied by Sentakukagaku-kyokai, 2-11-1 Shimomaruko Ohta-ku, Tokyo 146-8620, Japan) were added, and the suspension was homogenised with an UltraTurrax® blender. The cotton swatches were placed flat on a horizontal metal surface. 250µl of the carbon/gelatine suspension was pipetted onto the centre of each cotton swatch. The soiled cotton swatches were allowed to dry at room temperature overnight.

For this test the detergent composition was as described above and with the following surfactants and pH:

LAS: 0.4g per liter
Nonionic: 0.2g per liter
pH: 9.5

Enzyme detergency benefit results:

	Savinase® at 0.8 mg/l	Savinase® at 1.9 mg/l
No endo-glucanase	3.7	7.6
Endo-glucanase at 75 µg/l	16.6	
Endo-glucanase at 190 µg/l		17.7

The enzyme concentrations are specified here in terms of the equivalent concentration of the pure, catalytically active enzyme proteins, in order to avoid ambiguity resulting from activity assay procedures. Standard biochemical techniques can be used to purify and characterise the enzymes.

A suitable protease can be obtained from samples of the commercial product Savinase® from Novozymes A/S or derived from *Bacillus lentus* NCIB 10309 as described in US 3,723,250.

The endo-glucanase is an endo-glucanase with anti-redeposition effect as defined above. A method for preparation of this enzyme is described in Example 2.

In this test the soil contains protein. The results show that the combinations of the endo-glucanase and Savinase® give a higher enzyme detergency benefit than Savinase® alone.

EXAMPLE 9: Wash test: Mannanase and endo-glucanase

The purpose of this test was to measure the enzyme detergency benefit of a mannanase alone and of a mannanase in combination with endo-glucanase on a soil that contains galactomannan.

For this test the soiled swatches were prepared as follows: Swatches of the pre-washed #2003 fabric, prepared as above, were soiled as follows. A solution of 667 mg of guar gum (Sigma®, G 4129) was prepared in 200ml tap water by warming and with stirring and 332 mg of carbon black ("carbon for detergency tests", supplied by Sentakukagaku-kyokai, 2-11-1 Shimomaruko Ohta-ku, Tokyo 146-8620, Japan) were added, and the suspension was homogenised with an UltraTurrax® blender. The cotton swatches were placed flat on a horizontal metal surface. 500µl of the carbon/guar gum suspension was pipetted onto the centre of each cotton swatch. The soiled cotton swatches were allowed to dry at room temperature overnight.

For this test the detergent composition was as described above and with the following surfactants and pH:

LAS: 0.4g per liter

Nonionic: 0.2g per liter

pH: 9.5

Enzyme detergency benefit results:

	Mannanase at 0.3 mg/l	Mannanase at 0.75 mg/l
No endo-glucanase	4.8	6.2
Endo-glucanase at 75 µg/l	16.2	
Endo-glucanase at 190 µg/l		12.6

The enzyme concentrations are specified here in terms of the equivalent concentration of the pure, catalytically active enzyme proteins, in order to avoid ambiguity resulting from activity assay procedures. Standard biochemical techniques can be used to purify and characterise the enzymes.

A suitable mannanase can be obtained from *Bacillus sp.*1633, disclosed in WO 99/64619. Preferred is the mannanase of SEQ ID NO: 2 of WO 99/64619.

The endo-glucanase is an endo-glucanase with anti-redeposition effect as defined above. A method for preparation of this enzyme is described in Example 2.

In this test the soil contains a galactomannan. The results show that the combinations of the endo-glucanase and mannanase give a higher enzyme detergency benefit than mannanase alone.

EXAMPLE 10: Wash test: Pectate Lyase and endo-glucanase

The purpose of this test was to measure the enzyme detergency benefit of a pectate lyase (BioPrep® L) alone and in combination with endo-glucanase on a soil that contains pectin.

BioPrep® is the trade name of a commercially available pectate lyase product produced by Novozymes A/S. BioPrep® is intended for use in cleaning of cotton fibres.

For this test the soiled swatches were prepared as follows: Swatches of the pre-washed #2003 fabric, prepared as above, were soiled as follows. A solution of 150 mg of pectin (Sigma®, P 9311) was prepared in 50ml tap water by warming and with stirring and 83 mg of carbon black ("carbon for detergency tests", supplied by Sentakukagaku-kyokai, 2-11-1 Shimomaruko Ohta-ku, Tokyo 146-8620, Japan) were added, and the suspension was homogenised with an UltraTurrax® blender. The cotton swatches were placed flat on a horizontal metal surface. 500µl of the carbon/pectin suspension was pipetted onto the centre of each cotton swatch. The soiled cotton swatches were allowed to dry at room temperature overnight.

For this test the detergent composition was as described above and with the following surfactants and pH:

LAS: 0.4g per liter
Nonionic: 0.2g per liter
pH: 9.5

Enzyme detergency benefit results:

	BioPrep® at 1 mg/l	BioPrep® at 2.5 mg/l
No endo-glucanase	-8.0	-5.1
Endo-glucanase at 75 µg/l	6.6	
Endo-glucanase at 190 µg/l		6.7

The enzyme concentrations are specified here in terms of the equivalent concentration of the pure, catalytically active enzyme proteins, in order to avoid ambiguity resulting from activity assay procedures. Standard biochemical techniques can be used to purify and characterise the enzymes.

A suitable pectate lyase can be obtained from *Bacillus licheniformis*, as described in US 6,124,127.

The endo-glucanase is an endo-glucanase with anti-redeposition effect as defined above. A method for preparation of this enzyme is described in Example 2.

In this test the soil contains a pectin. The results show that the combinations of the endo-glucanase and pectate lyase give a higher enzyme detergency benefit than the pectate lyase alone.

EXAMPLE 11: Wash test: Lipex® and endo-glucanase

The purpose of this test was to measure the enzyme detergency benefit of a lipase (Lipex®) alone and in combination with endo-glucanase on a soil that contains triglyceride oils.

Lipex® is the trade name of a commercially available lipase product produced by Novozymes A/S. Lipex® is intended for use in detergents for laundry and hard surface cleaning.

For this test the soiled swatches were prepared as follows: Swatches of the pre-washed #2003 fabric, prepared as above, were soiled as follows. A suspension of 1.5 g of oat flakes (rolled, toasted oats, containing approximately 7% fats by weight, e.g. from Fællesindkøb I/S, 2605 Brøndby, Denmark) was prepared in 500ml tap water by stirring and heating to boiling and homogenising with an UltraTurrax® blender and then cooling. To 100ml

of this suspension 166 mg of carbon black ("carbon for detergency tests", supplied by Sentakukagaku-kyokai, 2-11-1 Shimomaruko Ohta-ku, Tokyo 146-8620, Japan) were added, and the suspension was homogenised with an UltraTurrax blender. 25ml of corn oil (refined corn oil, for household use, 100% oil by weight) were added and the mixture was homogenised with an UltraTurrax® blender. The cotton swatches were placed flat on a horizontal metal surface. 100µl of the carbon/oil suspension was pipetted onto the centre of each cotton swatch. The soiled cotton swatches were allowed to dry at room temperature overnight.

For this test the detergent composition was as described above and with the following surfactants and pH:

LAS: 0.4g per liter
Nonionic: 0.2g per liter
pH: 9.5

Enzyme detergency benefit results:

	Lipex® at 140 µg/l	Lipex® at 710 µg/l
No endo-glucanase	-1.7	-4.0
Endo-glucanase at 75 µg/l	46.4	
Endo-glucanase at 190 µg/l		53.9

The enzyme concentrations are specified here in terms of the equivalent concentration of the pure, catalytically active enzyme proteins, in order to avoid ambiguity resulting from activity assay procedures. Standard biochemical techniques can be used to purify and characterise the enzymes.

The lipase can either be obtained from samples of the commercial product Lipex® from Novozymes A/S or derived from suitable *Humicola lanuginosa* variants as described in WO 00/60063.

The endo-glucanase is an endo-glucanase with anti-redeposition effect as defined above. A method for preparation of this enzyme is described in Example 2.

In this test the soil contains a triglyceride oil. The results show that the combinations of the endo-glucanase and Lipex® give a higher enzyme detergency benefit than Lipex® alone.

EXAMPLE 12: Tests for endo-glucanase with anti-redeposition effect

Two enzymes were evaluated by the tests for "endo-glucanase with anti-redeposition effect". These were:

The enzyme that can be prepared as described in Example 2 above ("Example 2 enzyme"), and

Carezyme, i.e. the 43 kD cellulase derived from *Humicola insolens*.

Results:

	deltaOD from test for endo-glucanase activity	Anti-redeposition effect
Example 2 enzyme	0.4	10
Carezyme	>1	0

The results show that the Example 2 enzyme is an anti-redeposition endo-glucanase, i.e. an endo-glucanase with anti-redeposition effect according to the invention, and that Carezyme is not.

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0-1	**Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared using	PCT-EASY Version 2.92 (updated 01.11.2003)
0-2	**International Application No.**	PCT/DK 03/00844
0-3	**Applicant's or agent's file reference**	10383.204-WO

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	page	3
1-2	line	22-27
1-3	**Identification of Deposit**	
1-3-1	Name of depositary institution	DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
1-3-2	Address of depositary institution	Mascheroder Weg 1b, D-38124 Braunschweig, Germany
1-3-3	Date of deposit	25 January 1999 (25.01.1999)
1-3-4	Accession Number	DSMZ 12648
1-4	**Additional Indications**	NONE
1-5	**Designated States for Which Indications are Made**	all designated States
1-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE

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